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

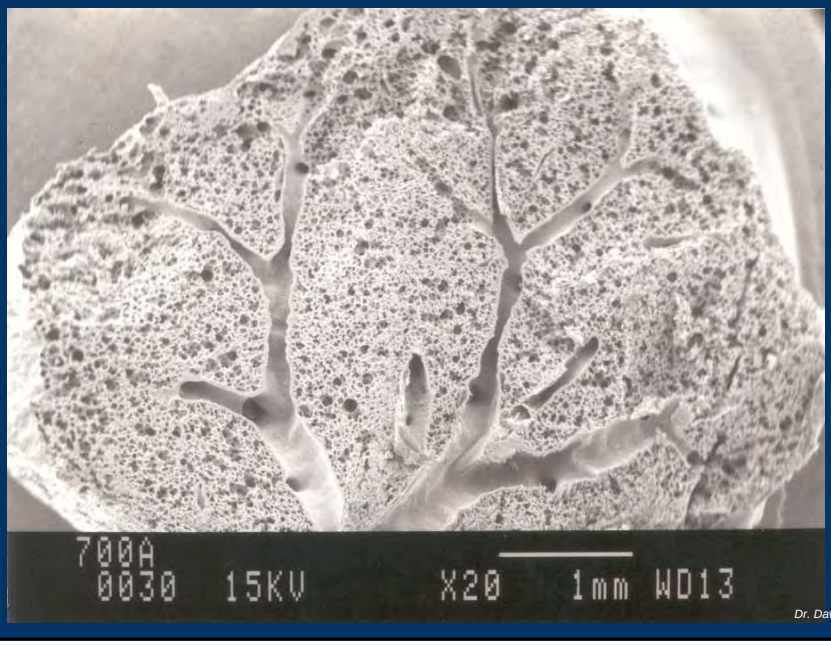
- Lung structure and particle deposition
- Pulmonary bioassay as a measure of lung toxicity- Hazard Assessment
- Pulmonary bioassay with Fine/Nanoscale TiO_2 dots and rods; Fine/Nanoscale Quartz particles, and Fine/Nanoscale ZnO particles
- Impacts of Particle Surface Coatings
- Summary

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

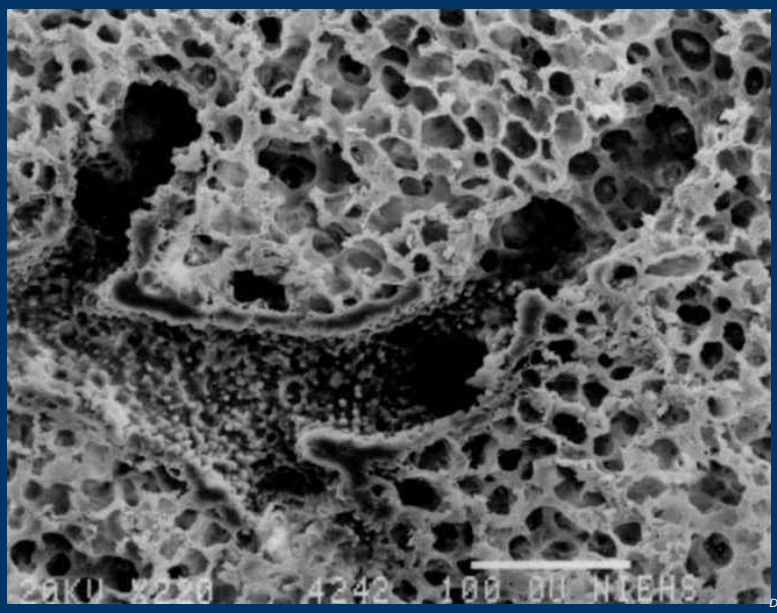
Dr. Dav d Warhe t
3 of 79

Rat Lung Microdissection



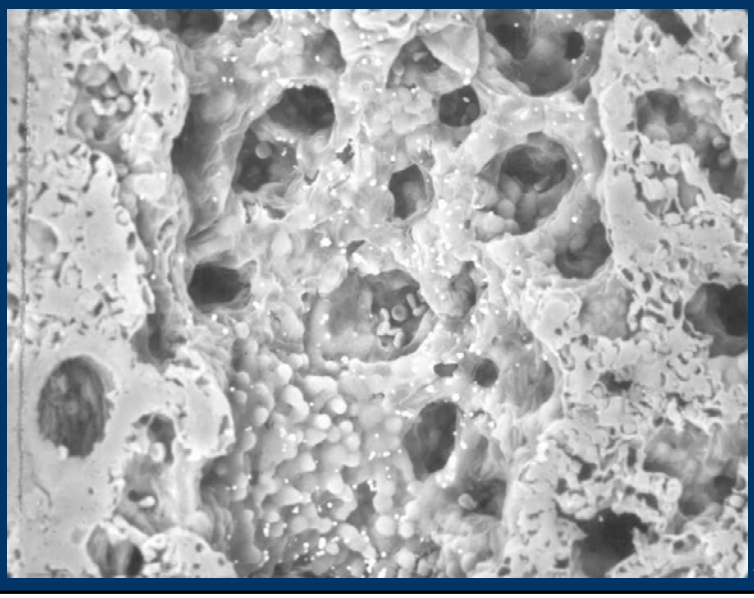
Dr. Dav d Warhe t
4 of 79

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



Dr. Dav d Warhe t
5 of 79

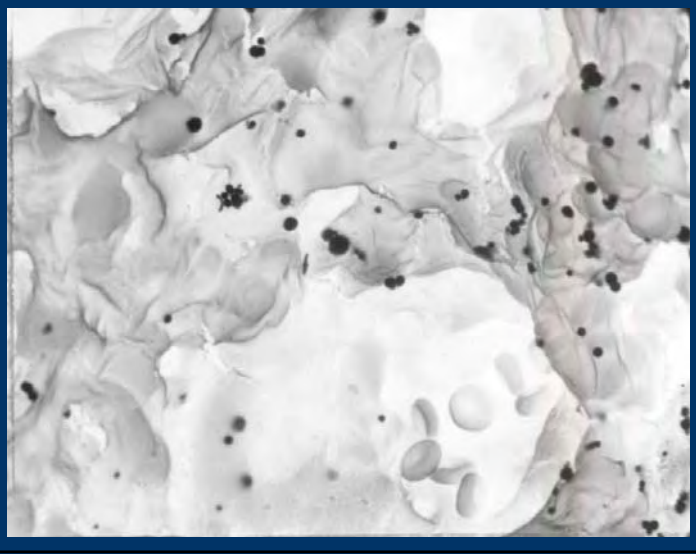
Iron Particle Deposition at Bronchoalveolar Junction



Dr. Dav d Warhe t
6 of 79

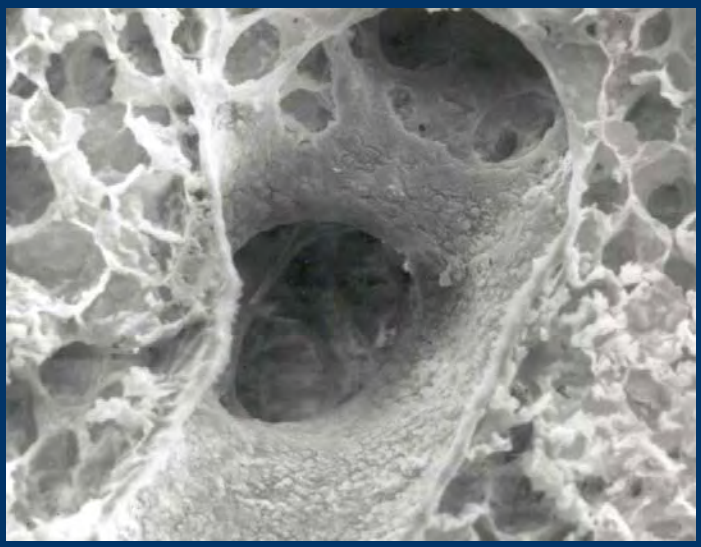
Iron Particle Deposition at Bronchoalveolar Junction

(Backscatter Image)



Dr. Dav d Warhe t
7 of 79

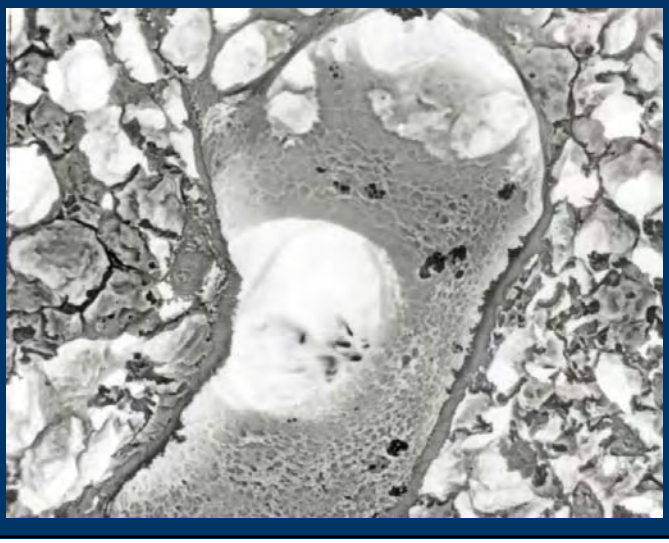
Alveolar Macrophage Clearance of Inhaled Iron Particles



Dr. Dav d Warhe t
8 of 79

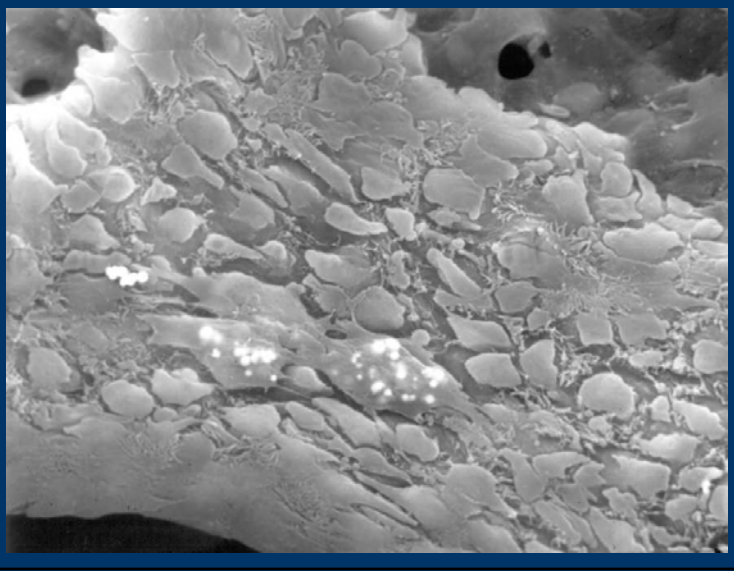
Alveolar Macrophage Clearance of Inhaled Iron Particles

(Backscatter Image)



Dr. Dav d Warhe t
9 of 79

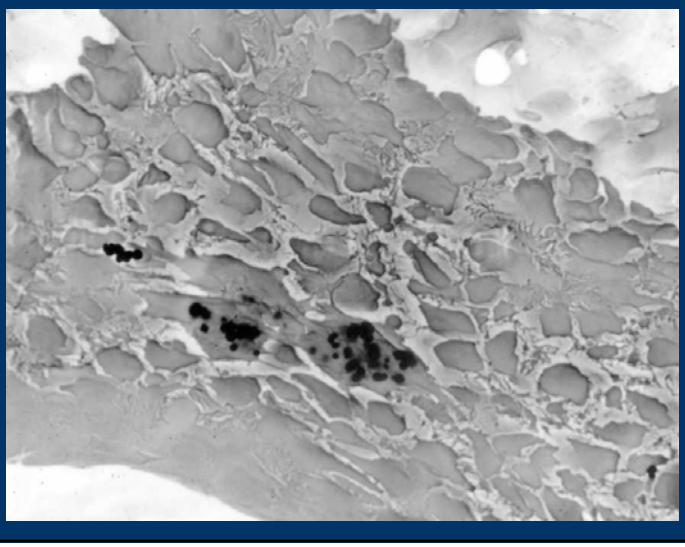
Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis



Dr. Dav d Warhe t
10 of 79

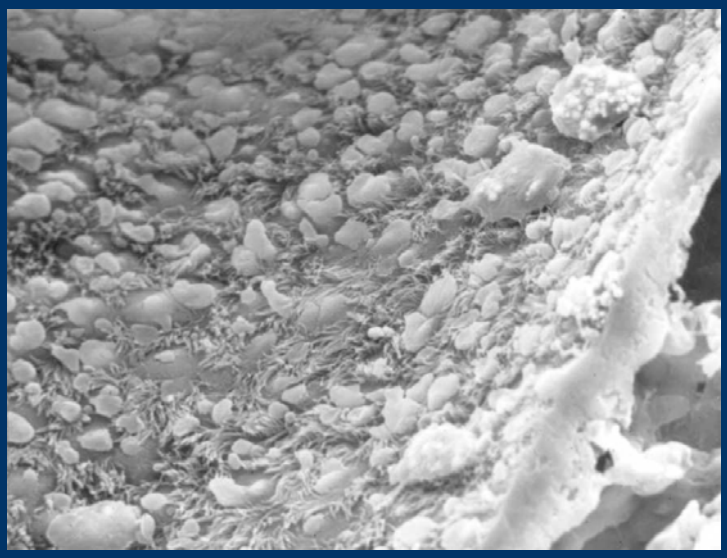
Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis

(Backscatter Image)



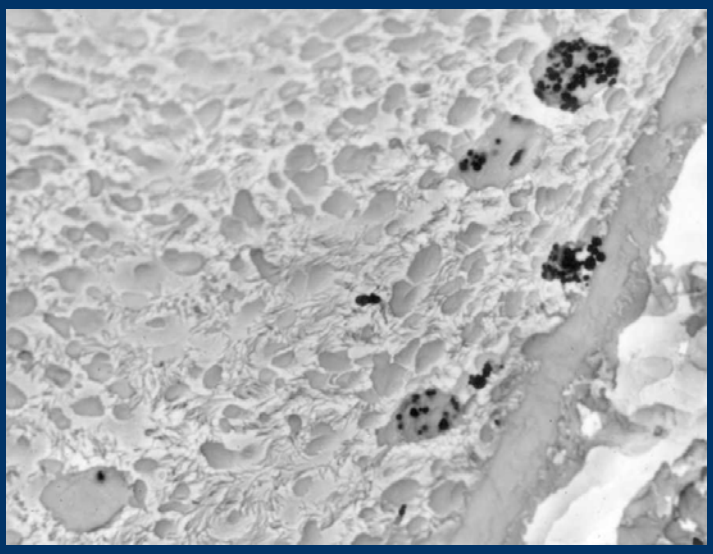
Dr. Dav d Warhe t
11 of 79

Clearance of Iron Particles on the Airway Mucociliary Escalator



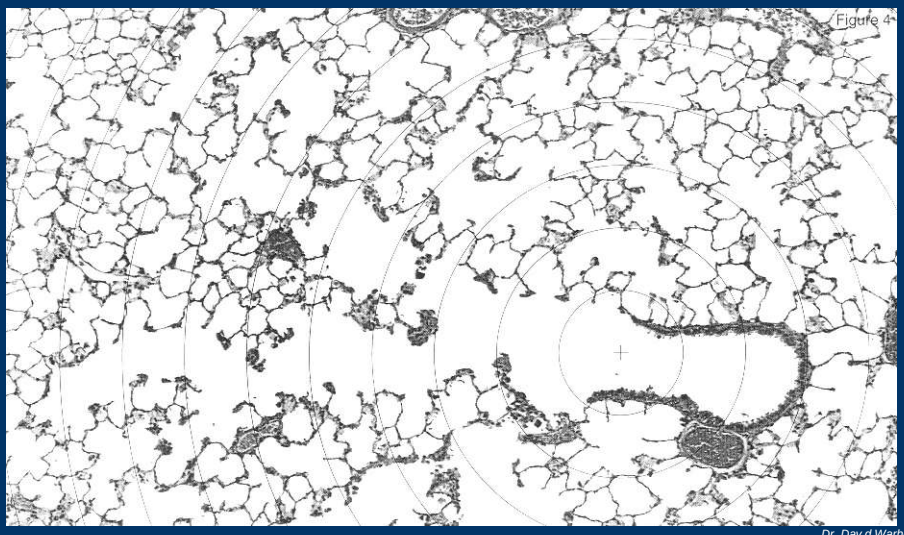
Dr. Dav d Warhe t
12 of 79

Clearance of Iron Particles on the Airway Mucociliary Escalator



Dr. Dav d Warhe t
13 of 79

Morphometry at Bronchoalveolar Junctions



Dr. Dav d Warhe t
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

Dr. Dav d Warhe t
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

Dr. Dav d Warhe t
16 of 79

The Key Issue: Risk

Health Risk is a product of

- **Hazard and Exposure**

Dr. Dav d Warhe t
17 of 79

Studies to Assess Pulmonary Hazards to Nanoparticulates

Dr. Dav d Warhe t
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

Dr. Dav d Warhe t
19 of 79

Pulmonary Bioassay Components

Bronchoalveolar Lavage Assessments

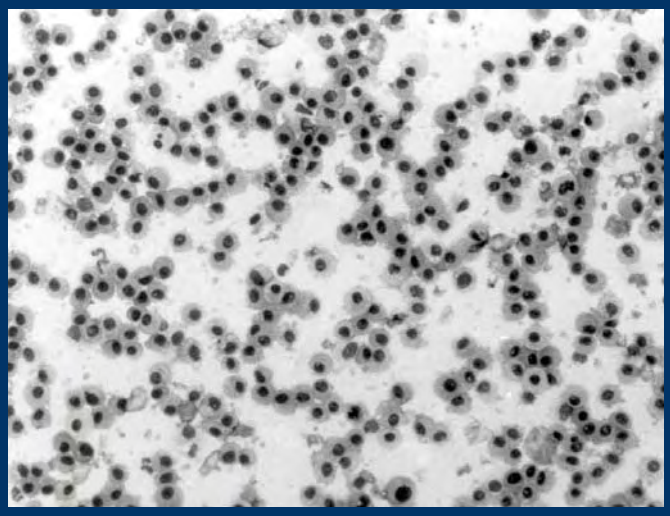
Lung Inflammation & Cytotoxicity
- Cell Differential Analysis
- BAL Fluid Lactate Dehydrogenase (cytotoxicity)
- BAL Fluid Alkaline Phosphatase (epithelial cell toxicity)
- BAL Fluid Protein (lung permeability)

Lung Tissue Analysis

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

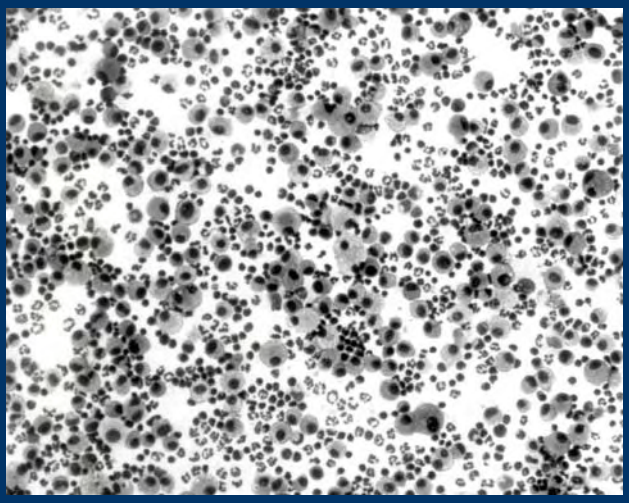
Dr. Dav d Warhe t
20 of 79

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



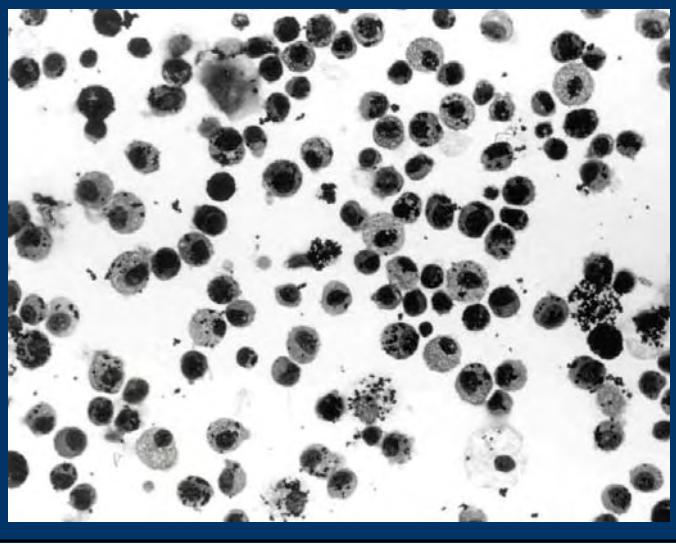
Dr. Dav d Warhe t
21 of 79

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



Dr. Dav d Warhe t
22 of 79

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



Dr. Dav d Warhe t
23 of 79

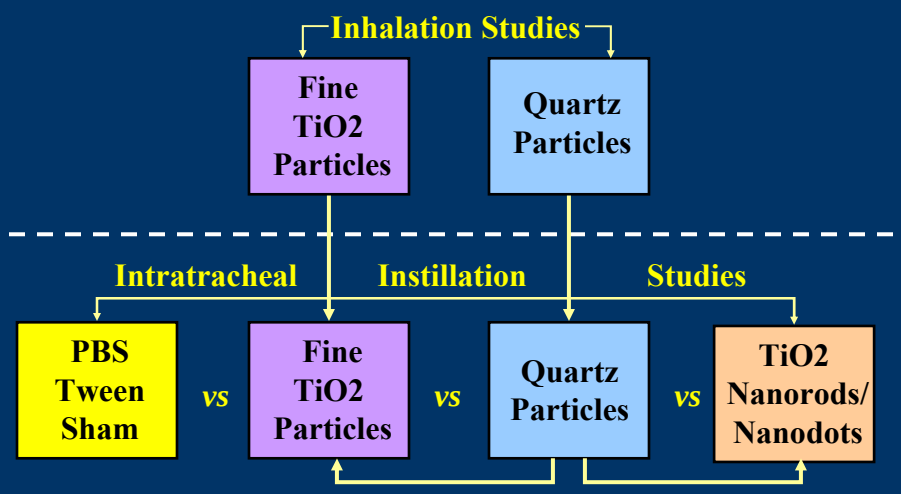
Use of Bronchoalveolar Lavage, Cell Proliferation, and Histopathology to Assess the Lung Toxicity of Particulate samples

(BALF = Bronchoalveolar Lavage Fluid Analysis)

Parameter	Indicator
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

Dr. Dav d Warhe t
24 of 79

Pulmonary Bioassay Bridging Studies



Dr. Dav d Warhe t
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

Dr. Dav d Warhe t
26 of 79

TiO_2

Dr. Dav d Warhe t
27 of 79

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

Dr. Dav d Warhe t
28 of 79

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

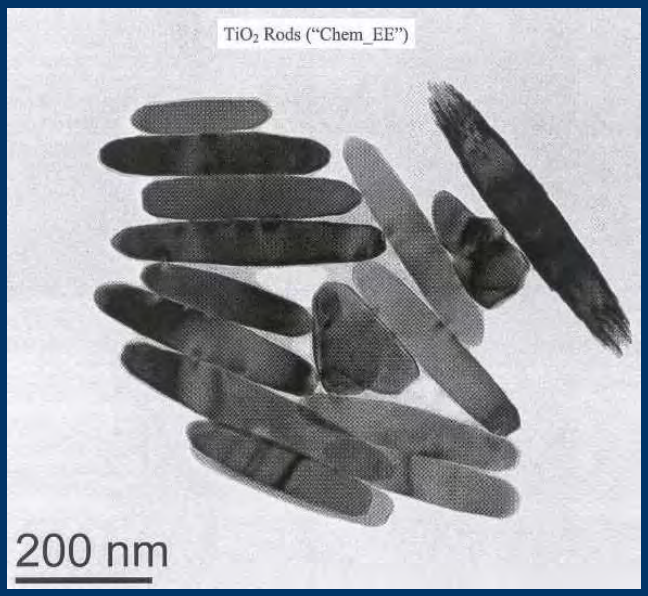
24 hr | 1 wk | 1 mo | 3 mo

Dr. Dav d Warhe t
29 of 79

TiO2 Nanoscale Dots

Dr. Dav d Warhe t
30 of 79

TiO2 Nanoscale Rods



Dr. Dav d Warhe t
31 of 79

TiPure® R-100

Dr. David Warheit
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**

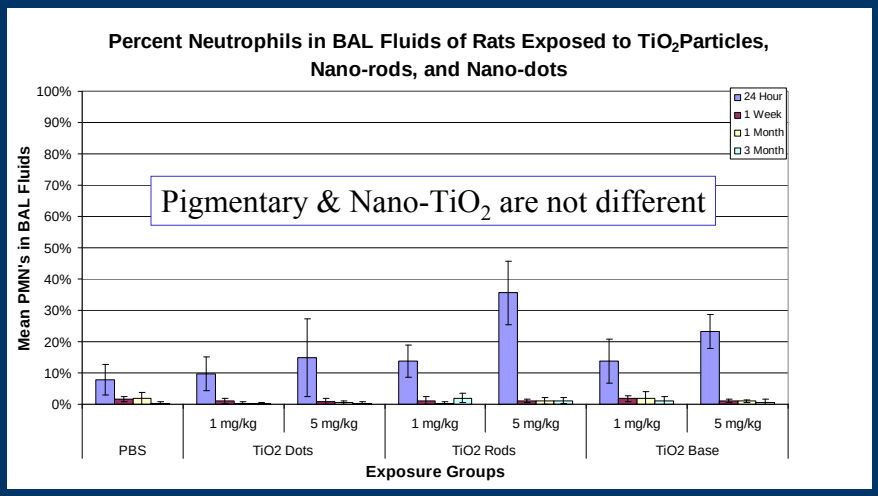
Dr. Dav d Warhe t
33 of 79

RESULTS

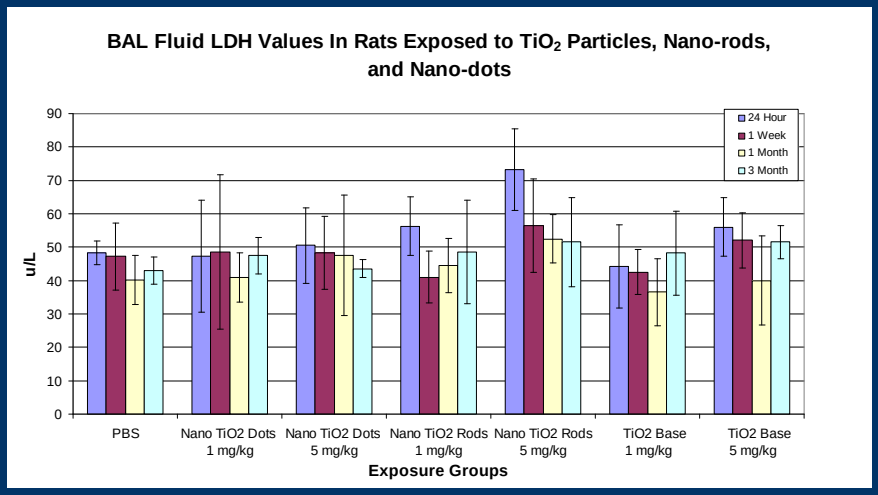
Biomarkers =
Pulmonary Inflammation
Pulmonary Cytotoxicity

Dr. Dav d Warhe t
34 of 79

Collaborative Studies with Rice University: TiO_2



Dr. Dav d Warhe t
35 of 79

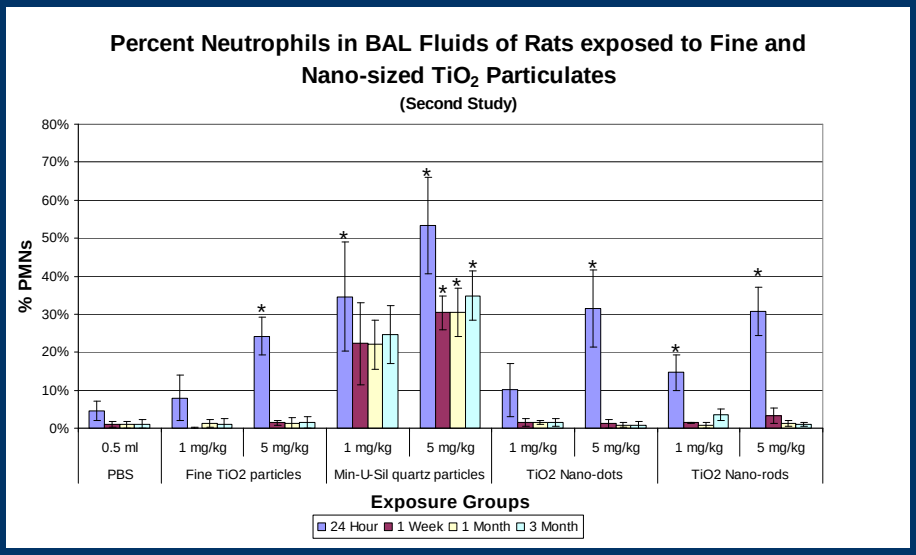


Dr. Dav d Warhe t
36 of 79

Second Nanoscale TiO2 Study

Dr. Dav d Warhe t
37 of 79

Pulmonary Inflammation

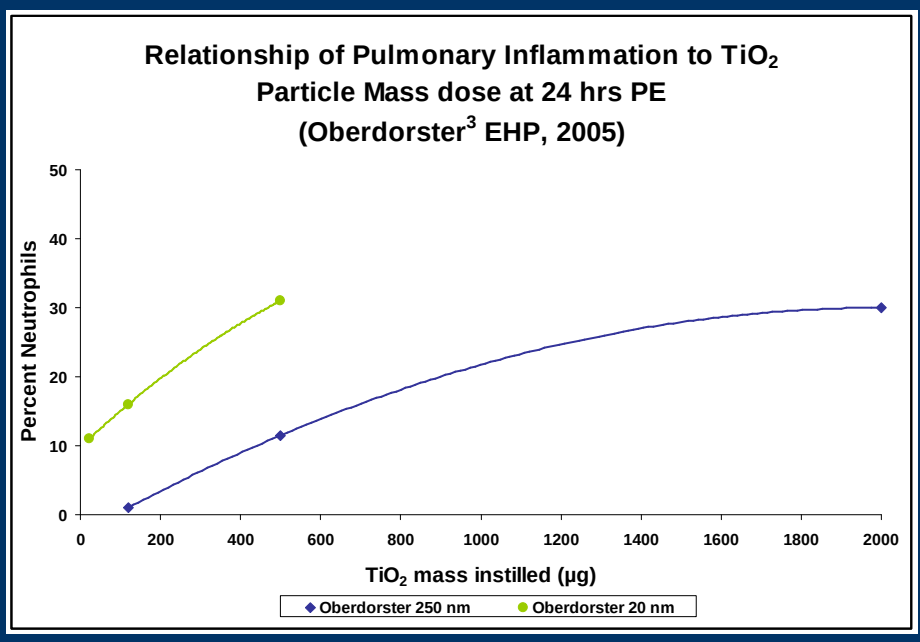


Dr. Dav d Warhe t
38 of 79

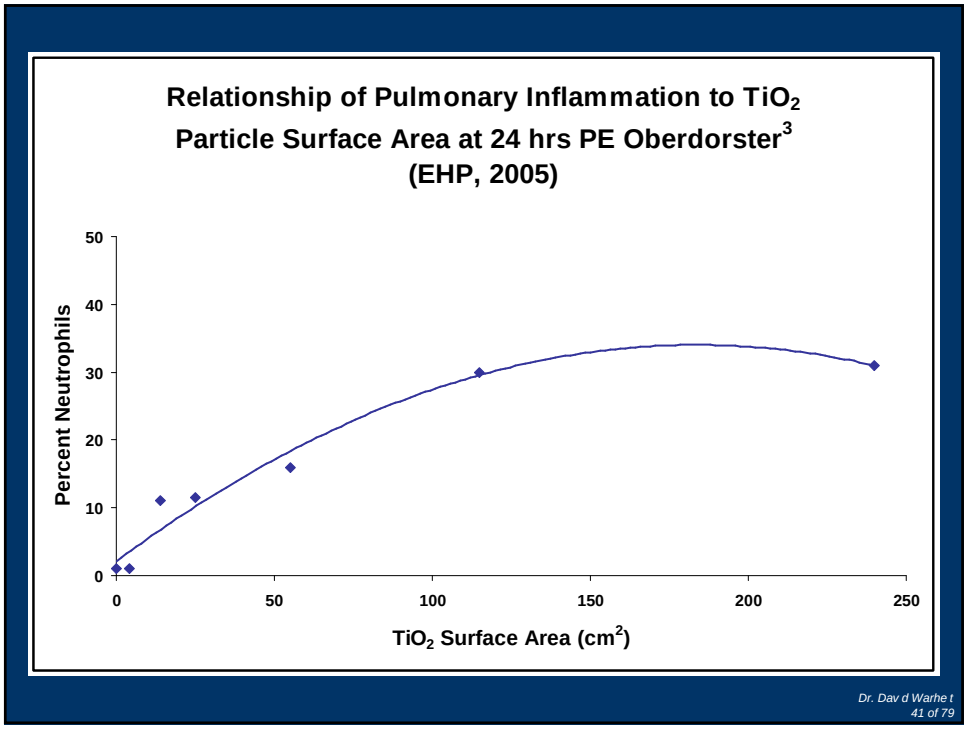
Characterization of Nanoscale TiO_2 and Quartz Particles

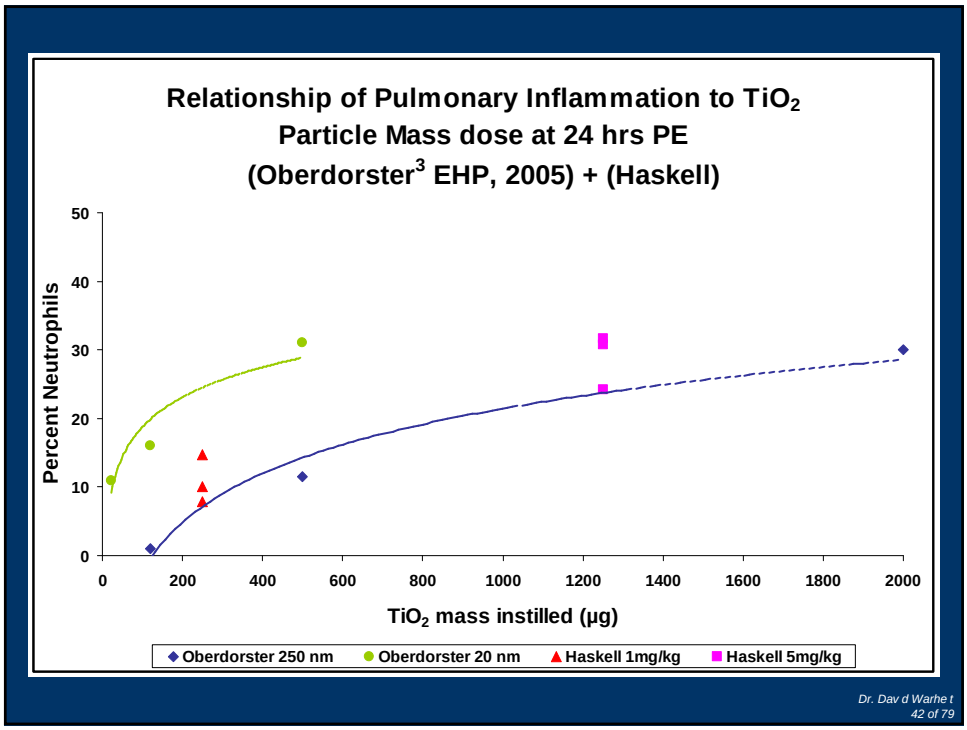
	XRD	particle size	Surface Area
• **Fine TiO_2**	rutile	d_{50} = 300 nm	**6.0 m^2/g**
• **TiO_2 Nanorods**	anatase	length= 90 - 233 nm width = 20 – 35 nm	**26.5 m^2/g**
• **TiO_2 Nanodots**	anatase	d_{50} = 6 nm	**169.4 m^2/g**
• **Min-U-Sil**	αQ	d_{50} = 1.3 µm	**4.0 m^2/g**

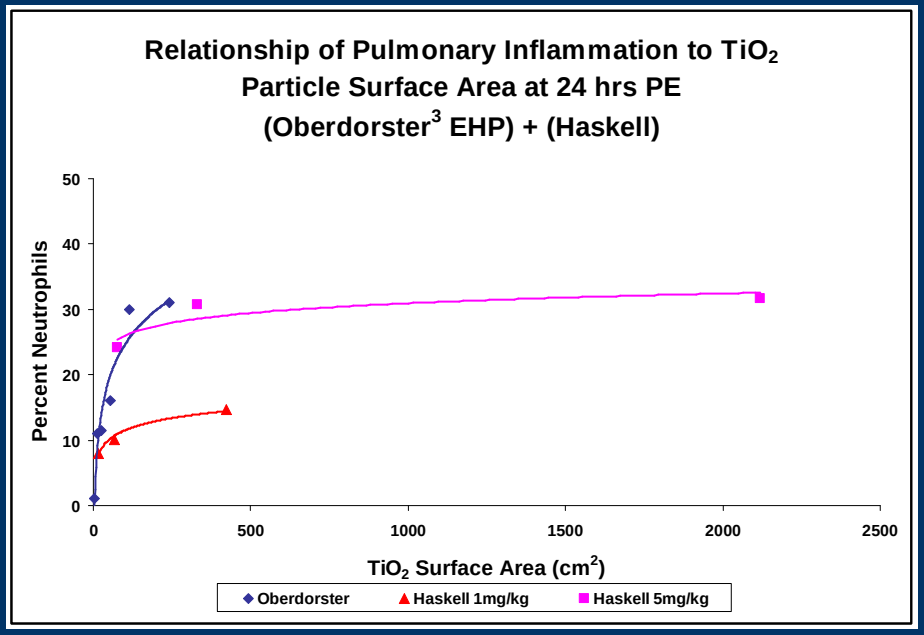
Dr. Dav d Warhe t
39 of 79



Dr. Dav d Warhe t
40 of 79


Relationship of Pulmonary Inflammation to TiO2
Particle Surface Area at 24 hrs PE Oberdorster3
(EHP, 2005)
Percent Neutrophils
0
10
20
30
40
50
0
50
100
150
200
250
TiO2 Surface Area (cm2)
Dr. David Warheit
41 of 79


Relationship of Pulmonary Inflammation to TiO2
Particle Mass dose at 24 hrs PE
(Oberdorster3 EHP, 2005) + (Haskell)
Percent Neutrophils
0
10
20
30
40
50
0
200
400
600
800
1000
1200
1400
1600
1800
2000
TiO2 mass instilled (µg)
Oberdorster 250 nm
Oberdorster 20 nm
Haskell 1mg/kg
Haskell 5mg/kg
Dr. David Warheit
42 of 79



Dr. Dav d Warhe t
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?

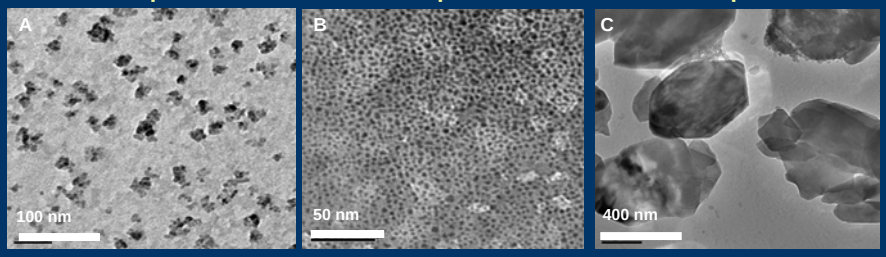
Dr. Dav d Warhe t
44 of 79

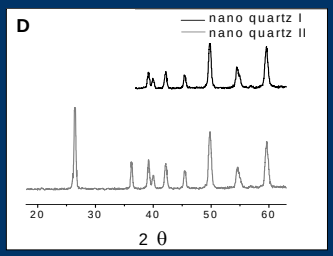
Nanoscale Quartz

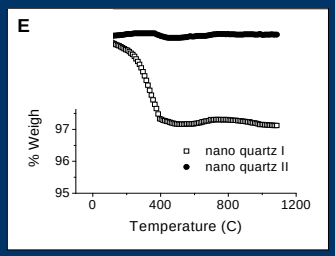
Dr. Dav d Warhe t
45 of 79

Physicochemical Characterization of Quartz Particulates

nano quartz I | nano quartz II | fine quartz







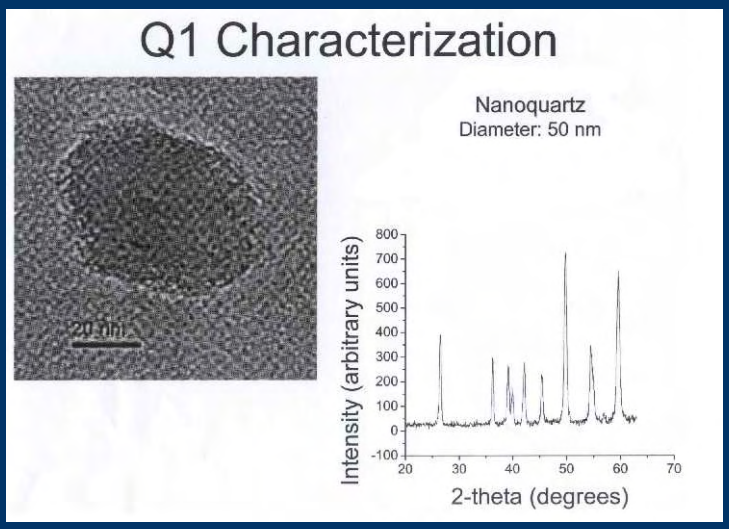
Dr. Dav d Warhe t
46 of 79

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%

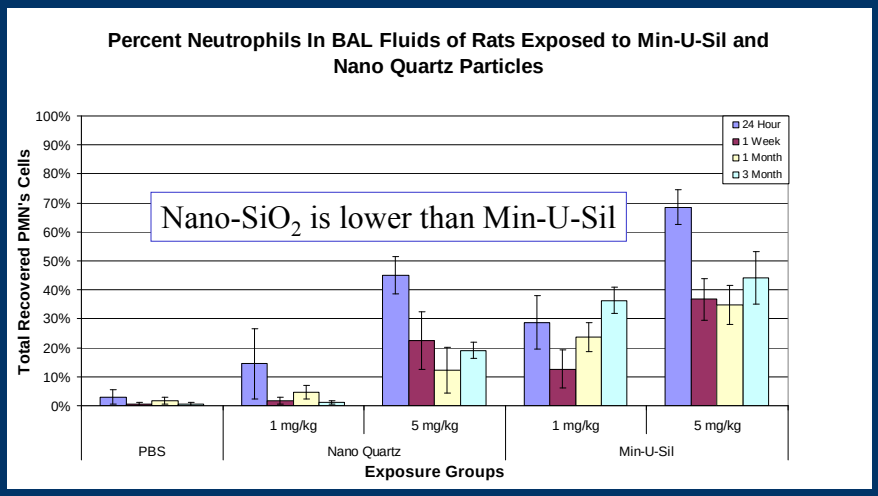
Dr. Dav d Warhe t
47 of 79

Nanoscale Quartz Particles

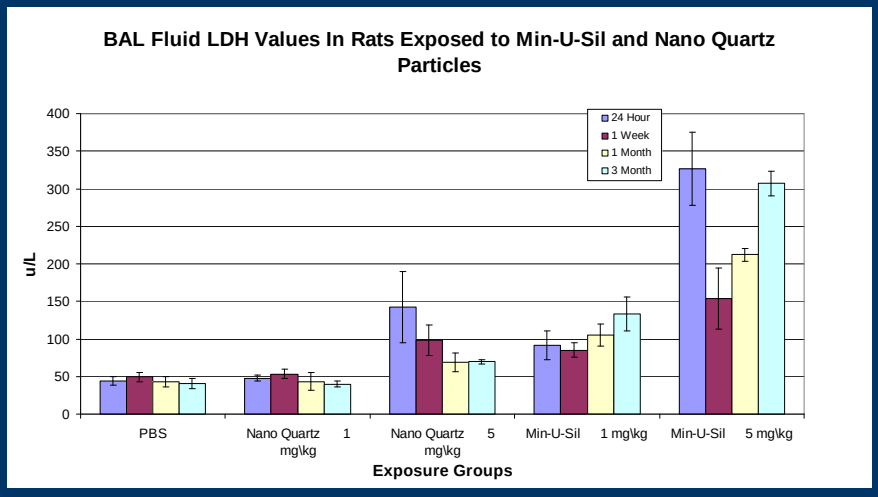


Dr. Dav d Warhe t
48 of 79

Preliminary Collaborative Studies with Rice University: SiO_2



Dr. Dav d Warhe t
49 of 79



Dr. Dav d Warhe t
50 of 79

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**

Dr. Dav d Warhe t
51 of 79

Second Nanoscale Quartz Study

Dr. Dav d Warhe t
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)

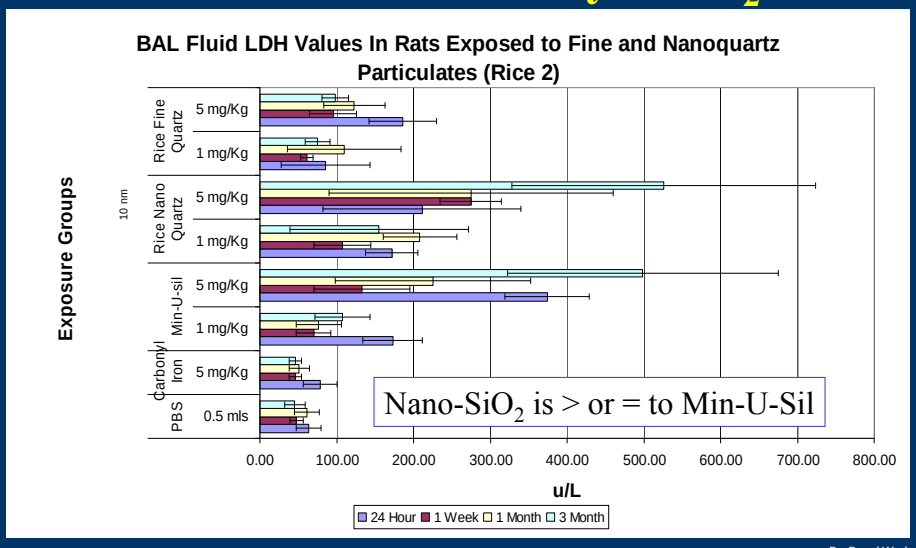
Instillation Exposure

Postexposure Evaluation via BAL and Lung Tissue

24 hr | 1 wk | 1 mo | 3 mo

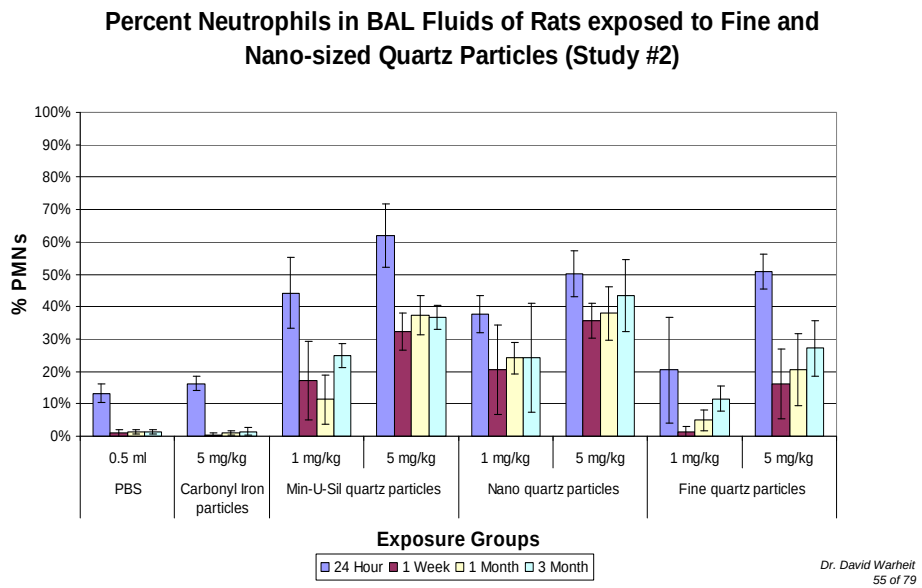
Dr. David Warheit
53 of 79

Follow-up Collaborative Studies with Rice University: SiO_2



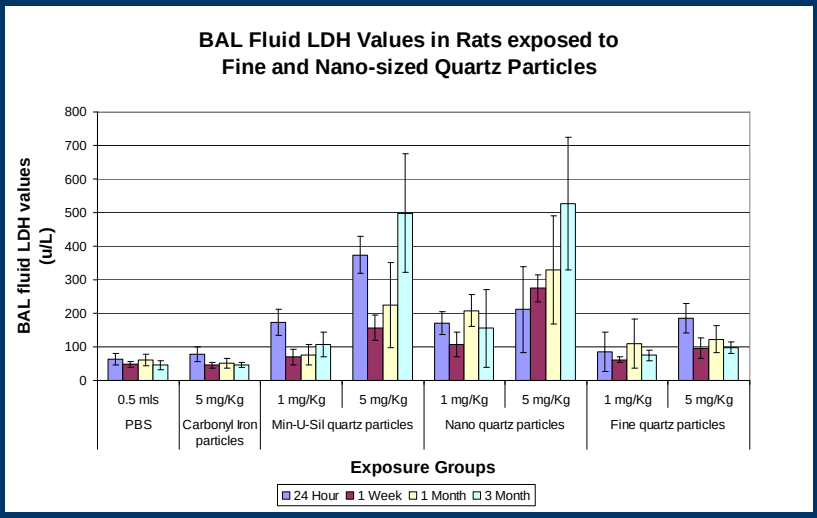
Dr. David Warheit
54 of 79

Pulmonary Inflammation



Dr. David Warheit
55 of 79

BAL Fluid LDH Values (cytotoxicity)



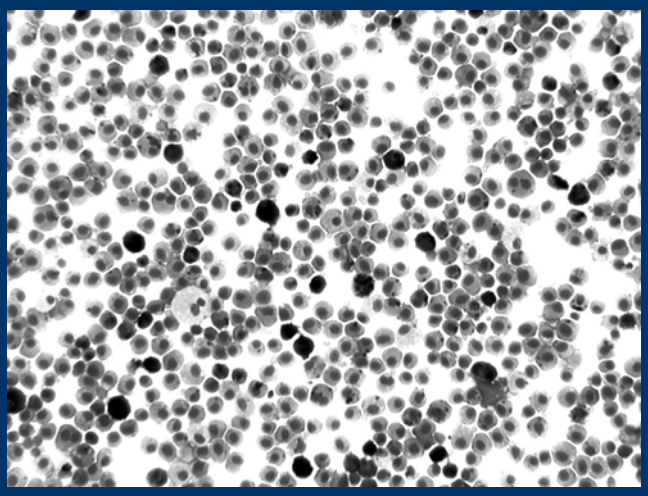
Dr. Dav d Warhe t
56 of 79

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**

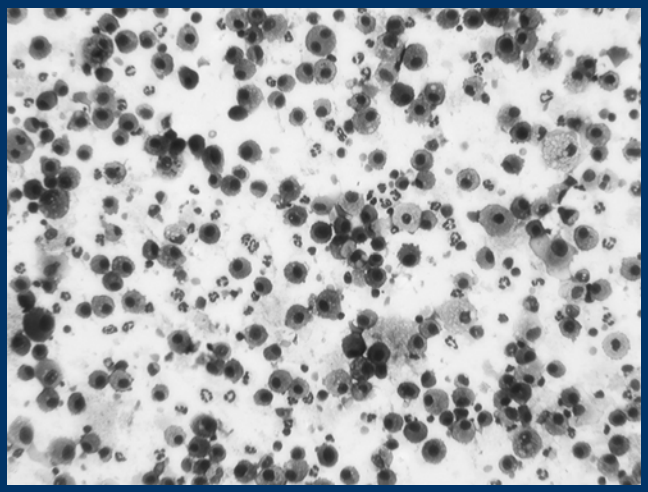
Dr. Dav d Warhe t
57 of 79

CI – 2B-3M – 2aab – 20x



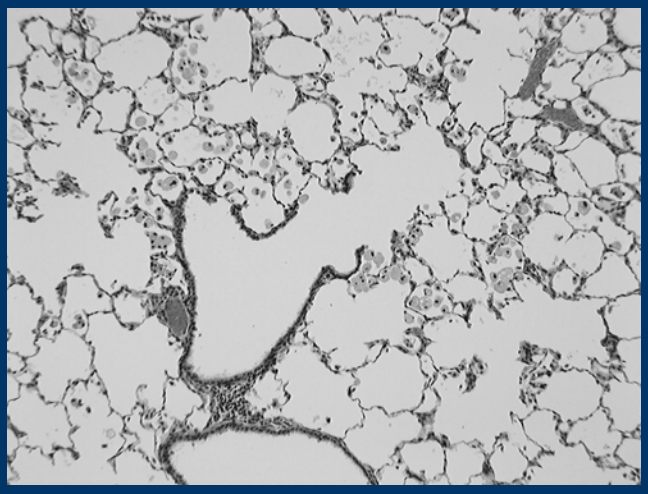
Dr. Dav d Warhe t
58 of 79

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



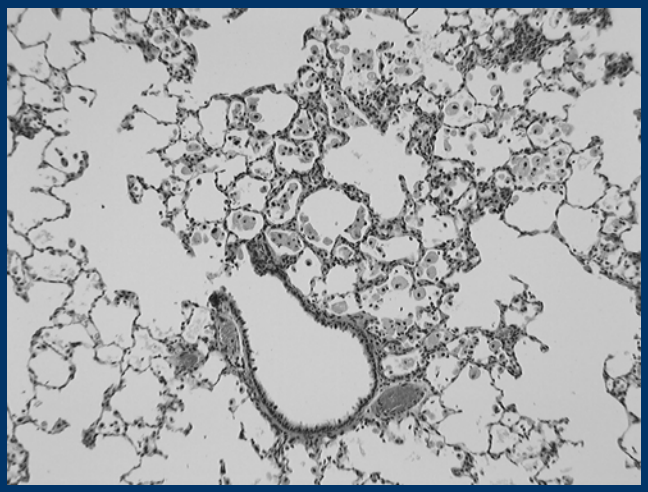
Dr. Dav d Warhe t
59 of 79

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



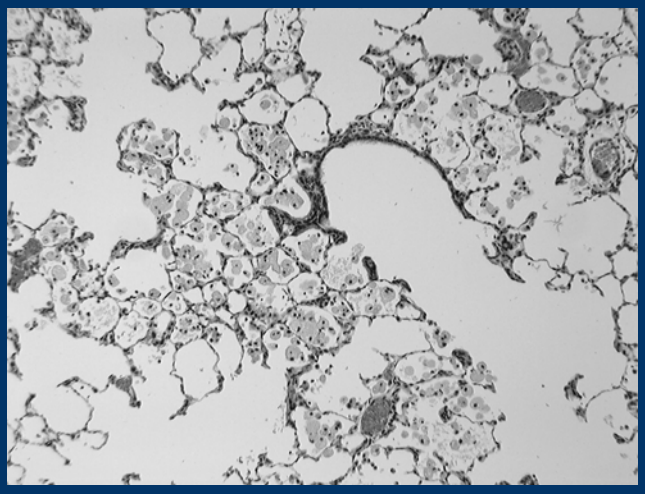
Dr. Dav d Warhe t
60 of 79

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



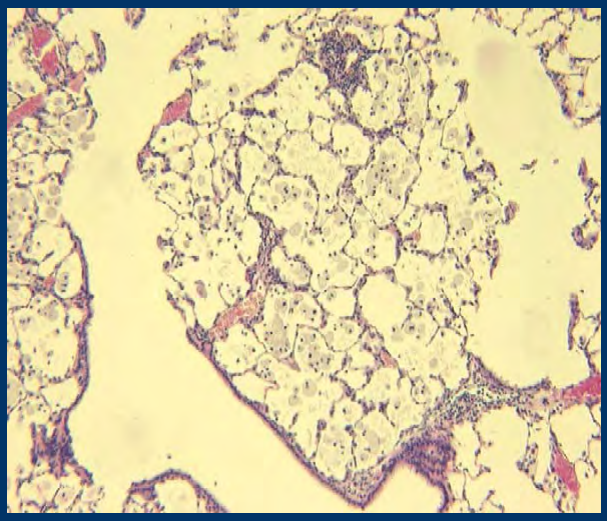
Dr. Dav d Warhe t
61 of 79

NanoQ-4B-3M-5



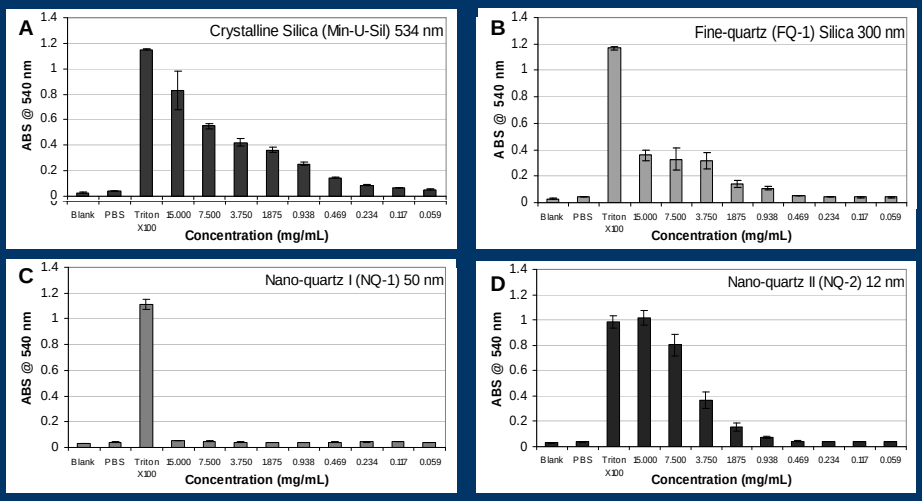
Dr. Dav d Warhe t
62 of 79

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



Dr. David Warheit
63 of 79

Hemolytic Potential of Quartz Samples (Surface Reactivity)



Dr. David Warheit
64 of 79

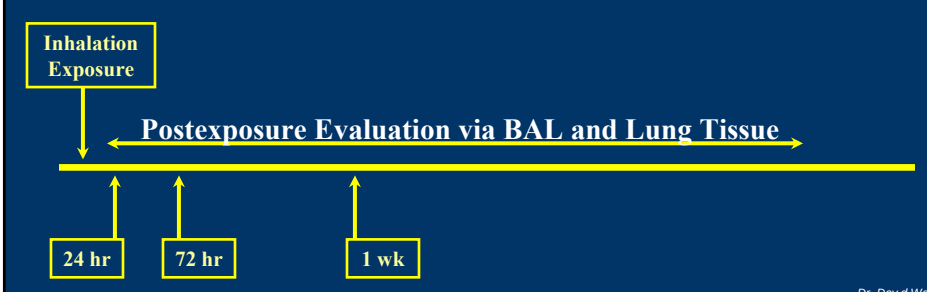
Summary of α-Quartz Results

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

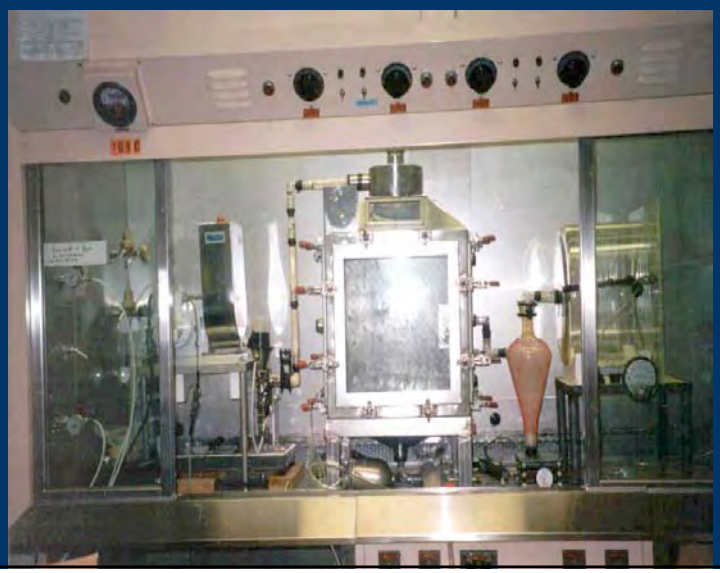
Protocol for Fine and Nanoscale ZnO Pulmonary Bioassay Studies

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



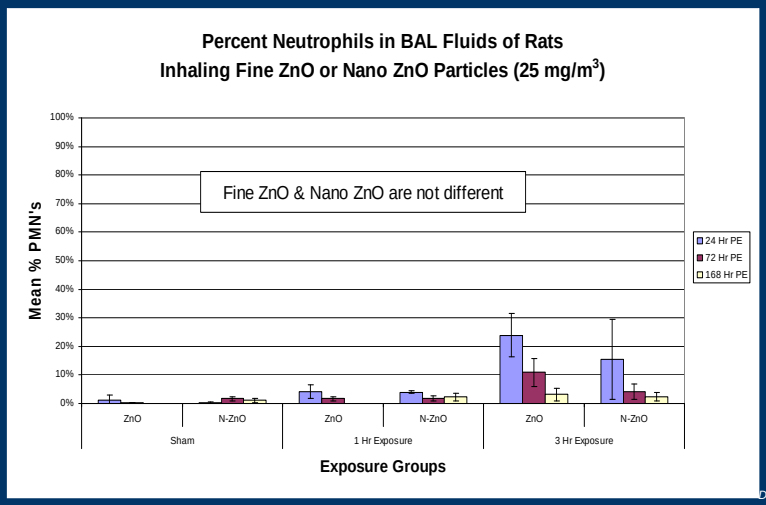
Dr. Dav d Warhe t
67 of 79

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

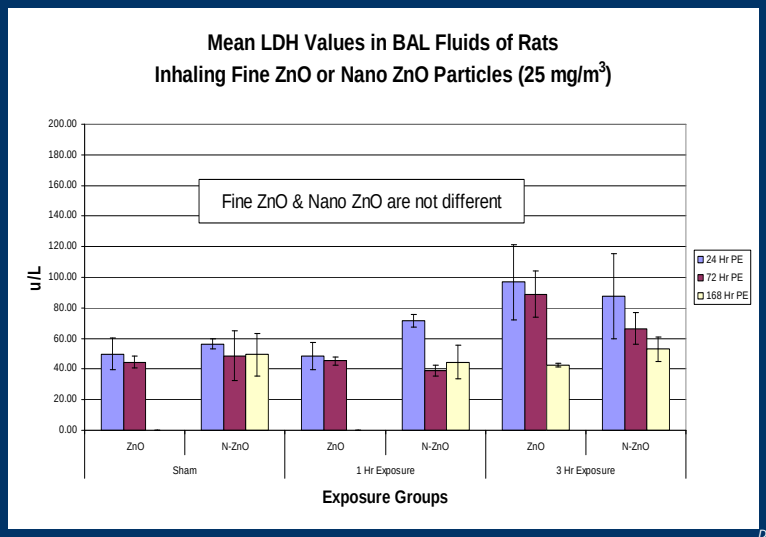
Dr. Dav d Warhe t
68 of 79

Preliminary Studies with Fine and Nano Zinc Oxide particles



Dr. David Warheit
69 of 79

Preliminary Studies with Fine and Nano Zinc Oxide particles



Dr. David Warheit
70 of 79

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

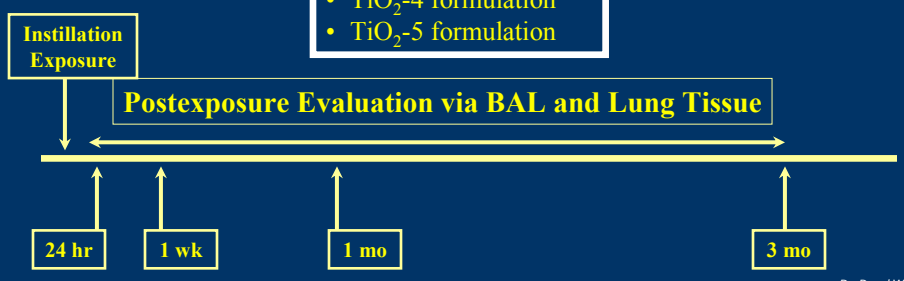
TiO2 V - 94% TiO2 - 3% alumina - 3% amorphous silica

Dr. Dav d Warhe t
73 of 79

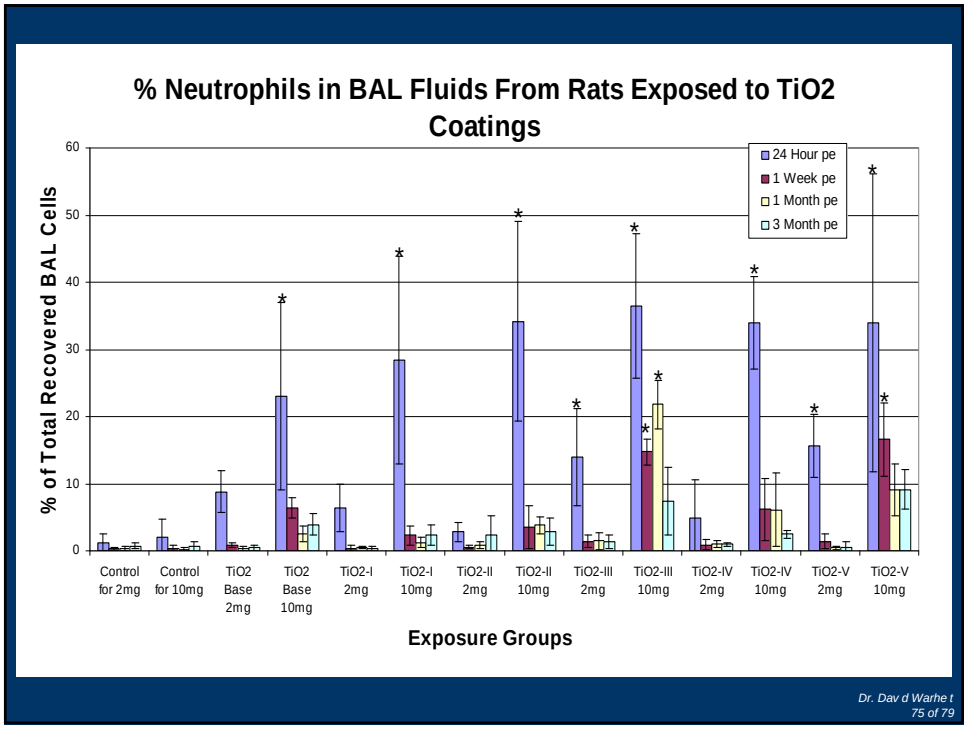
Protocol for TiO_2 Coatings Bioassay Study

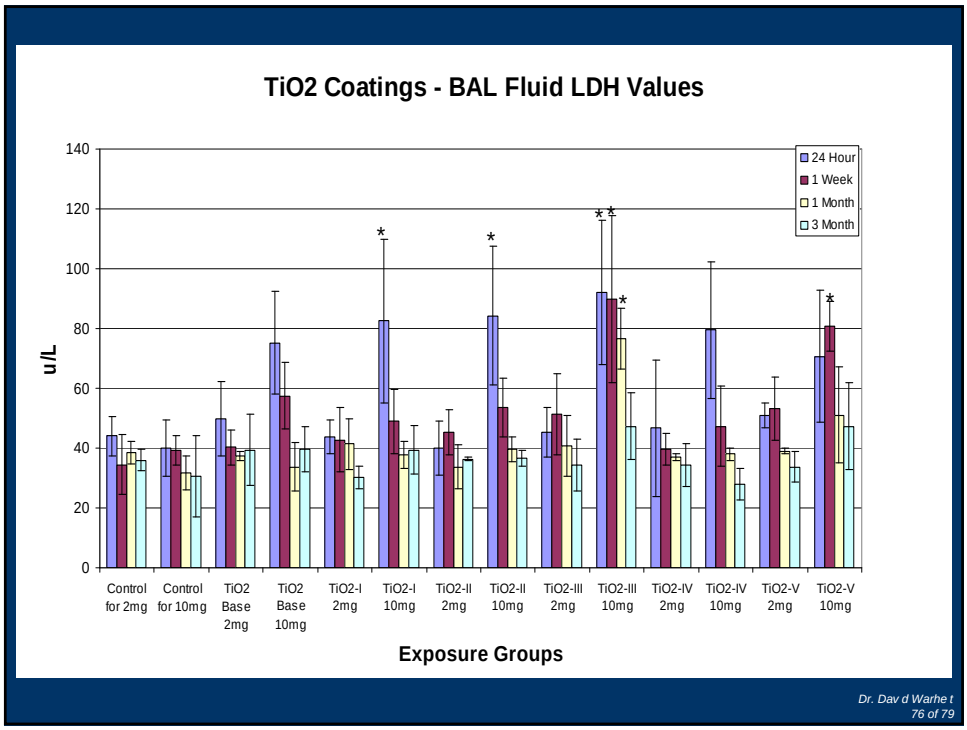
Instillation Study

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
0
10
20
30
40
50
60
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. Dav d Warhe t
75 of 79


TiO2 Coatings - BAL Fluid LDH Values
u/L
0
20
40
60
80
100
120
140
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. Dav d Warhe t
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.