Comments on the draft NIOSH Current Intelligence Bulletin: Evaluation of Health Hazard and Recommendations for Occupational Exposure to Titanium Dioxide

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Outline

- General conclusions regarding NIOSH CIB
- Chronic inhalation studies in rats
- Background on overload pulmonary effects – unique to rats
- Species differences in lung responses to particulates
- Epidemiological studies
- Pulmonary bioassay studies with nanoscale TiO_2 particles and implications
- Summary recommendations

General Conclusions I

- General document and pertinent literature review is for the most part, comprehensive
- However, many of the summaries are either misrepresented or the results misinterpreted.
- The authors ignore the critically relevant studies by Nikula et al., (1997, 2001) which demonstrate the fundamental differences in lung responses to low solubility dusts when comparing pulmonary responses or rats to humans and nonhuman primates.

General Conclusions II

- The authors draw the erroneous conclusion that since humans have slow dust clearance response, leading to particle overload, the human response might be similar to the rat response, possibly leading to lung tumors.
- At least 2 fundamental weaknesses to this argument:

  1) the epidemiology data is negative for lung cancer;

  2) the lung response of humans is different from rats – both in clearance kinetics, and with regard to inflammatory and pathological responses.

  Thus, the basis for conducting the risk assessment models is flawed.

General Conclusions III

- The authors put forth a weak and nonrelevant model suggesting that the human lung could respond to overload concentrations by citing the effects of humans to inhaled silica and asbestos. It is well known that the pulmonary responses to asbestos and silica differ from the responses to poorly soluble particulates (including TiO_2 particles) – with no known epidemiological relationship to lung cancer and no known evidence, from human lung pathology studies that, in the absence of smoking, exposure to poorly soluble particulates like coal dust or TiO_2 lead to lung cancer in humans.

General Conclusions IV

- Rats are uniquely sensitive to developing lung responses to poorly soluble particulates at particle overload concentrations. This response does not occur in ANY other species.

General Conclusions V

- Current Mode of Action Scenario for Development of Lung Tumors in Rats exposed to Overload concentrations of PSPs
- In rats→ chronic exposure to TiO_2 → Particle Overload → sustained inflammation → epithelial cell proliferation, septal fibrosis and fibroproliferative effects → metaplasia → mutations → lung tumors
- In humans → chronic exposure to PSP → Particle Overload → low degree of inflammation → little or no Fibrosis → no Mutations → No lung tumors

General Conclusions VI

- Poor Justification for the suggested RELs for pigment grade (1.5 mg/m^3) or ultrafine TiO_2 particles (0.1 mg/m^3) (John Tomenson)

- Throughout the CIB document, the authors fail to adequately characterize the crystal structures and particle sizes (physical characteristics) used by the investigators in the various studies.
- This is important because the document assumes that all of the ultrafine TiO_2 particles are similar or identical to the ultrafine P25 TiO_2 particles used in the Heinrich and Bermudez studies. In fact, not all ultrafine TiO_2 particles have the same crystal structure or particle size as P25 (80% anatase: 20% rutile)

General Conclusions VII

- Particle overload/surface area issues in Rat lung inflammatory/tumor responses are unique to that species and are not relevant for Humans.
- The Epidemiological evidence in humans exposed to TiO_2 particles is negative. In addition, there is no histopathological evidence that exposure to PSPs including TiO_2 produce lung tumors in humans.
- Ultrafine TiO_2 particles are comprised of different crystal structures (anatase or rutile) and different particle sizes and the suggestion that P25 is representative for all ultrafine TiO_2 particle-types is flawed.

Background – Important publications on Particle Effects in the Rat Lung

- Lee et al., Tox Appl Pharm. 79:179-192, 1985.
- Heinrich et al., Inhal Toxicol 7:533-556, 1995.
- Hext – Human & Exper Toxicol 13:700-715, 1994
- Warheit et al., Toxicol Appl Pharmacol 145: 10-22, 1997
- Carlton – Fundam Appl Toxicol 23:304-307, 1994
- Levy – In: Toxic and Carcinogenic Effects of Solid Particles, 1994
- Boorman et al. – Toxicological Pathology 24: 564-573, 1996

Important publications on Particle Effects in the Rat Lung (Cont)

- Warheit and Frame – JOEM, 2006 in press
- Vu – Inhalation Toxicol 8 (suppl) 181-191, 1996.
- PCRARM, Presidential Commission on Risk Assessment and Risk Management (1997)
- Nikula et al., Fundam. Appl Toxicol 37: 37-53, 1997.
- Nikula et al. Environ Health Perspec 109: 311-318, 2001.
- Warheit et al. Toxicological Sciences, in press 2006

Important publications on Particle Effects in the Rat Lung (Cont)

- Tran et al., Inhal Toxicol 12:1113-1126, 2000.
- Bermudez et al., Toxicol Sci. 70:86-97, 2002.
- Bermudez et al., Toxicol. Sci. 77:347-357, 2004

Important Epidemiological publications on Exposure Effects in TiO2 Workers

- Chen and Fayerweather – J Occup Med 30:937-942, 1988.
- Boffetta et al., Scand J Work Environ Health 27:227-232, 2001.
- Fryzek et al, J Occup Environ Med 45:400-409, 2003
- Boffetta et al., (Cancer Causes and Control (2004)

Inhalation Toxicity studies- fine and ultrafine TiO_2 particles

- **Lee et al.**, (1985) fine TiO_2 ~ 300 nm
  - M and F rats – 0, 10, 50 and 250 mg/m3 for 2 yrs
  - 10 mg/m3 - minor effects
  - 50 mg/m3 - various effects + fibrosis + no tumors
  - 250 mg/m3 – various effects + fibrosis + ~ 25 % benign tumors + PKC
- **Heinrich et al.**, (1995) ultrafine TiO_2 P-25 (10-40 nm)

  - F Wistar rats – DEEP 7 mg/m3, CB 11.6 mg/m3, P-25 10 mg/m3 – 2 yrs + 6 months
  - Lung Tumors: DEEP 22/100; CB 39/100; P-25 32/100

Hext – Current Perspectives on Particulate Induced Pulmonary Tumours

- Chronic exposure to insoluble particulates can lead to the development of pulmonary tumors in rats but not in other rodent species.
- Prerequisite – overloading of normal alveolar macrophage mediated mechanisms.
- Inflammation → epithelial hypertrophy and/or hyperplasia and squamous metaplasia.
- Persistence of these effects leads to lung tumors.

Warheit et al. – Inhalation of high concentrations of low toxicity dusts results in impaired pulmonary clearance mechanisms and persistent inflammation

- Male rats exposed to TiO_2 or carbonyl iron particles for 4 weeks at 5, 50 and 250 mg/m^3 and evaluated through 6 months postexposure.

- Exposures to 250 mg/m^3 produced sustained inflammation, enhanced proliferation of pulmonary cells, impairment of particle clearance, deficits in macrophage function and macrophage aggregates at sites of particle deposition.

Carlton – "Proliferative Keratin Cyst" – a lesion in the lungs of rats following chronic exposure to para-aramid fibrils

- Workshop of pathologists to reach a diagnostic descriptive consensus on cystic keratinzing pulmonary lesions produced in rats by para-aramid fibrils and TiO2 particles.

- All participants agreed that the cystic lesions were not malignant neoplasms. A majority considered the lesions to be nontumorous "proliferative keratin cysts" (PKC). A minority (3/13) considered the lesions to be benign tumors.

Levy – Squamous lesions associated with chronic exposure by inhalation of rats to p-aramid fibrils and to titanium dioxide: Findings of a pathology workshop

- Report of the same workshop – implications of the PKC for humans.
- None of the pathologists had observed these lesions in humans.
- These lesions generally considered to be unique to rats.

Boorman et al. Classification of cystic keratinizing squamous lesions of the rat lung: Report of a workshop

- Workshop of pathologists reached a consensus on classification of these unique pulmonary tissues responses in response to particle overload exposures in the rat lung.

Warheit and Frame - Characterization and reclassification of TiO_2- related pulmonary lesions (JOEM, in press 2006)

- OBJECTIVE: Utilizing current diagnostic criteria, the manuscript summarized the microscopic review of 16 proliferative squamous lesions, previously diagnosed as cystic keratinizing squamous cell carcinoma, in the lungs of rats from a two-year inhalation study with pigment-grade titanium dioxide particles.
- RESULTS: Unanimous agreement was reached as to the diagnosis of each of the lesions. Two of the lesions were diagnosed as squamous metaplasia and one as a poorly-keratinizing squamous cell carcinoma. The remaining 13 lesions were diagnosed as non-neoplastic pulmonary keratin cysts

Vu – Use of hazard and risk information in risk management decisions: Solid particles and fibers under EPA's TSCA and EPCRA

- Since the enactment of EPCRA, EPA has received many petitions to delist chemicals from the TRI. After a careful review of pertinent health information on TiO_2, (particles 1-3 μm diameter) EPA issued a final rule deleting this substance from the list of toxic chemicals under section 313 of Title III of SARA.
- With regard to human health and the environment, EPA has determined that TiO_2 poses a low risk of acute respiratory effects.

Vu - continued

- TiO_2 is of low acute toxicity by inhalation.
- There is insufficient evidence of chronic respiratory effects, carcinogenicity, and heritable mutations.
- The single positive result at the time of the EPA review (Lee et al., 1985), in which an increase in lung tumors was observed, occurred in rats exposed via inhalation to high concentrations that may have overwhelmed clearance mechanisms of the lung.
- Along with the negative carcinogenic responses from multiple studies by various routes of exposure + the negative mutagenicity data leads to an overall weight of evidence determination that there is not sufficient evidence to show that TiO_2 will cause or can reasonably be expected to cause cancer in humans (USEPA, 1988).

PCRARM – Presidential Commission on Risk Assessment and Risk Management (1997) Final Report, Volume 2

- "There are ...cases ... where rodent tumor responses have been shown to be irrelevant to humans or may occur at doses far exceeding any recognized human exposures..."
- The PCRARM specifically identified TiO_2 as one such chemical because observed rodent tumor responses associated with exposure to TiO_2 are not relevant to human risk.

Nikula et al., - Lung tissue responses and sites of particle retention differ between rats and Cynomolgus monkeys exposed chronically to diesel exhaust and coal dust.

- Study compared the anatomical pattern of particle retention and the lung tissue response of rats and monkeys exposed chronically (24 months) to high occupational concentrations of poorly soluble particles

Nikula-1 (cont)

- Rats retained greater portion of particles in alveolar ducts vs. monkeys
- Monkeys retained greater portion of particles in interstitium vs. rats.
- Rats but not monkeys had significant alveolar epithelial hyperplastic, inflammatory and septal fibrotic responses to the retained particles.
- Authors concluded that particle retention patterns and tissue reactions in rats may not be predictive for primates at high conc.

Nikula et al. Influence of exposure conc. or dose on the distribution of particulate material in rat and human lungs

- Study used morphometry to assess particle retention in histologic sections from rats and humans.
- Rats exposed 24 months to DEEP at 0.35, 3.5 or 7 mg soot/m3
- Humans – nonsmoking coal miners.
- Retained particle distribution within the lungs markedly different in rats vs. humans.

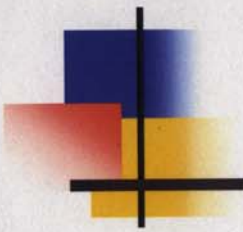
Nikula-2 (cont)

- In rats - chronically inhaled diesel soot is retained in alveolar regions.
- In humans - chronically inhaled particulate matter is retained primarily in interstitium.
- In humans – percentage of particles in the interstitium ↑ with ↑ dose
- Difference in distribution may account for species differences in lung response to particles.

Species Comparisons of Pulmonary Effects of Particle Overload in Rats vs Large Mammals

Classical attributes and sequelae of lung overload in rats	Rats	Dog, Monkey, and Man
Chronic pulmonary inflammation	Yes	Not certain
Hyperplasia of macrophages and epithelial cells	Yes	Not certain
Altered pulmonary clearance (overwhelms) macrophage mediated clearance	Yes	Probably not
Large pulmonary burdens of particles	Yes	Probably not
Increased interstitialization of deposited particles	Yes	Yes
Increased translocation of particles from lung to thoracic lymph nodes	Probably	Probably
Interstitial lung disease (fibrosis)	Yes	Yes but less severe
Production of lung tumors	Yes	No

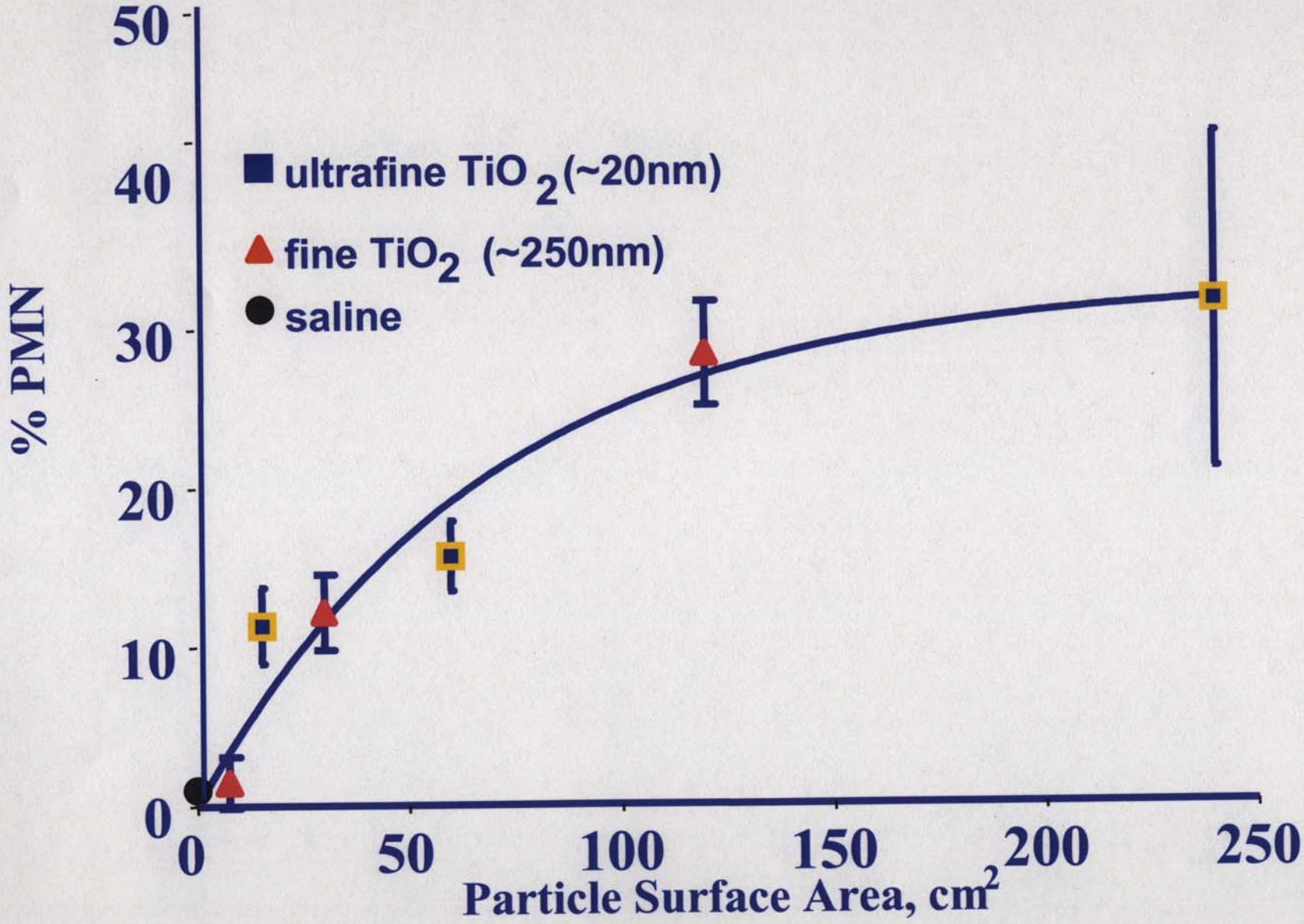
Tran et al., Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance.



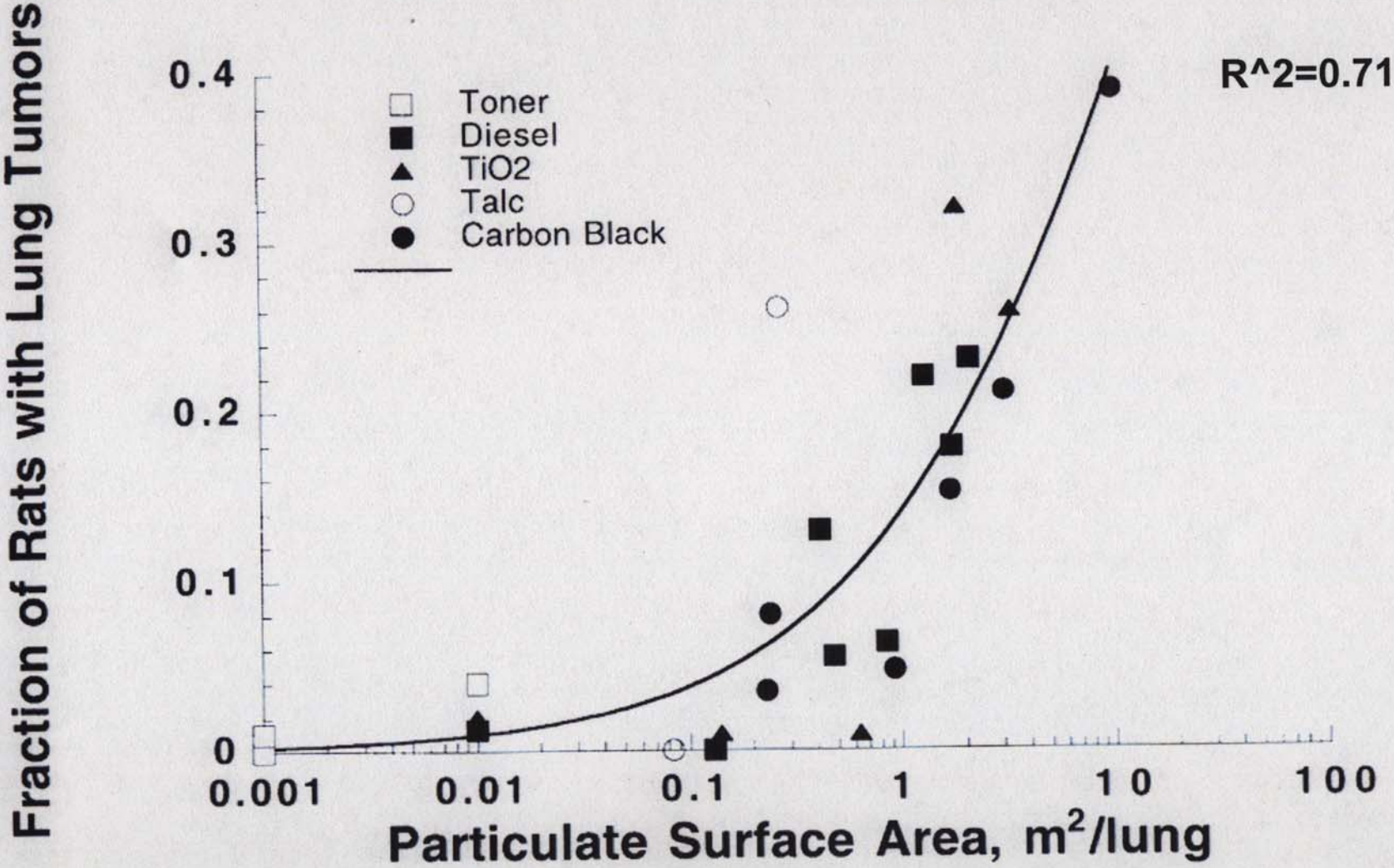
- Tested volumetric overload hypothesis- which predicts the impairment of particle clearance in terms of particle volume
- Evaluated exposures to TiO_2 and $BaSO_4$.
- Concluded that inflammation and translocation best described when the lung burden was expressed as total particle surface area.

Percent of PMN in BAL 24 hrs After Instillation of TiO_2 in Rats

Correlation with Particle Surface Area- Oberdorster,1994



Relationship between particle surface area and tumor formation in the rat



Adapted from data published by Driscoll (1996)

CIIT Inhalation Toxicity studies- fine and ultrafine TiO_2 particles

Bermudez et al., " Pulmonary Responses of Mice, Rats, and Hamsters to Subchronic Inhalation of Ultrafine Titanium Dioxide Particles" - 2004

- F rats, mice and hamsters exposed for 13 weeks to 0.5, 2.0 and 10 mg/m^3 – and evaluated over 1 yr pe.
- **In summary, there were significant species differences in lung responses to ufTiO_2- rats more severe inflammation, progressive epithelial and fibroproliferative lesions.**

Bermudez et al., 2002

- Pigment grade TiO_2 study – 0, 10, 50 and 250 mg/m^3
- The 10 mg/m^3 effect in rats exposed to ufTiO_2 was similar to the 50 mg/m^3 pigment-grade and not the 250 mg/m^3 pigment.

Take home points regarding the lung toxicity of fine and ultrafine TiO_2 particles

- Pulmonary lesions were most severe in rats, where progressive epithelial- and fibroproliferative changes were observed in the 250 mg/m^3 group. These epithelial proliferative changes were also manifested in rats as an increase in alveolar epithelial cell labeling in cell proliferation studies. Associated with these foci of epithelial proliferation were interstitial particle accumulation and alveolar septal fibrosis.

Take home points - II

- In summary, there were significant species differences in pulmonary response to inhaled pigment grade TiO_2 particles. Under conditions in which the lung pigment grade TiO_2 burdens were similar and likely to induce pulmonary overload, rats developed a more severe and persistent pulmonary inflammatory response than either mice or hamsters.

Take home points – III

- Rats also were unique in the development of progressive fibroproliferative lesions and alveolar epithelial metaplasia in response to 90 days of exposure to a high concentration of pigment grade TiO_2 particles.

Conclusions on Toxicology Studies in Rats with TiO_2 and PSPs

- Chronic exposures to overload concentrations of 250 but not 50 mg/m³ pigment-grade TiO_2 in rats produced benign lung tumors.
- Rats are uniquely sensitive to overload concentrations of poorly soluble particles and develop proliferative keratin cysts.
- Other rodent species do not develop lung tumors to high doses of PSP particle-types.
- The distribution and cellular responses to inhaled dusts in larger mammals is different when compared to rats.

Epidemiology Studies with Worker Exposure to TiO_2 Particles

1) Chen and Fayerweather; (1988) Epidemiologic study of workers exposed to titanium dioxide.
2) Boffetta et al., (2001) Exposure to titanium dioxide and risk of lung cancer in a population-based study from Montreal
3) Fryzek et al. (2003) A cohort mortality study among titanium dioxide manufacturing workers in the United States.

Epidemiology studies (cont.)

- Boffetta et al., (2004) – Mortality among workers employed in the titanium dioxide production industry in Europe.
- **The four epidemiology studies of large population cohorts in different parts of the world were negative for lung cancer in TiO_2-exposed workers.**

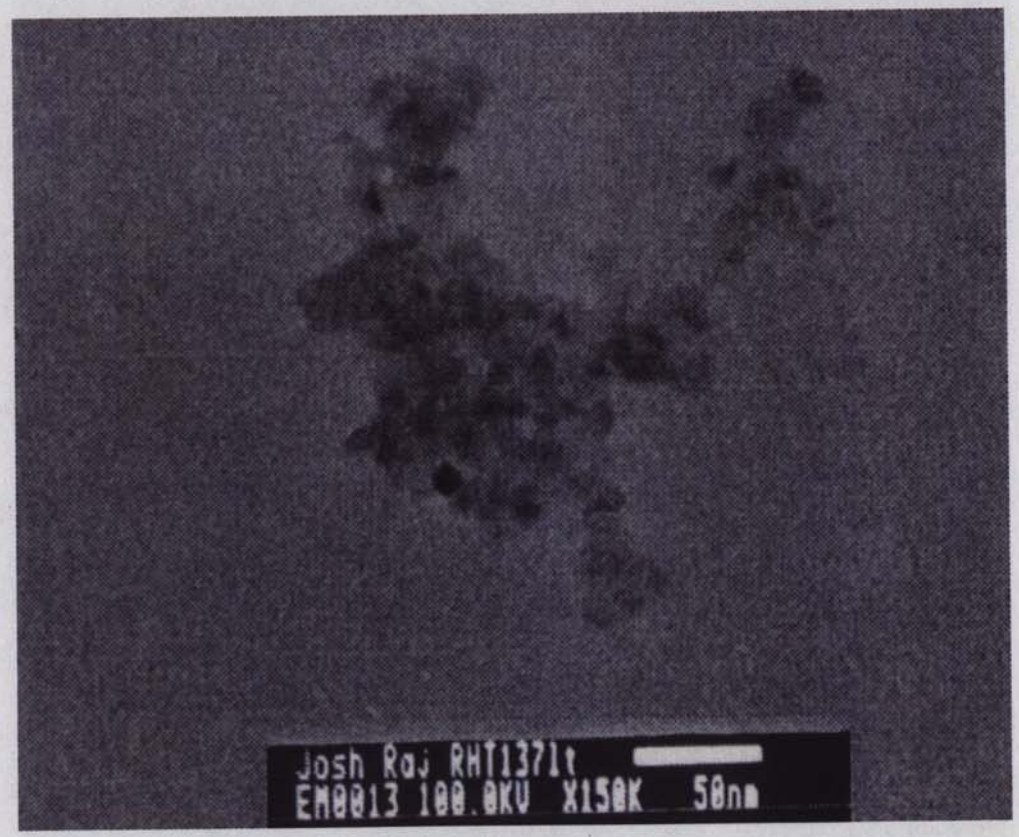
Pulmonary Bioassay Studies with Fine and Nanoscale TiO_2 Particulates

New Information - Particle Surface indices do not always correlate with Pulmonary Inflammation in Rats

- Pulmonary Instillation Studies with Nanoscale TiO_2 Rods and Dots in Rats: Toxicity is not dependent upon Particle Size and Surface Area – Warheit et al., in press, **Toxicological Sciences, 2006.**

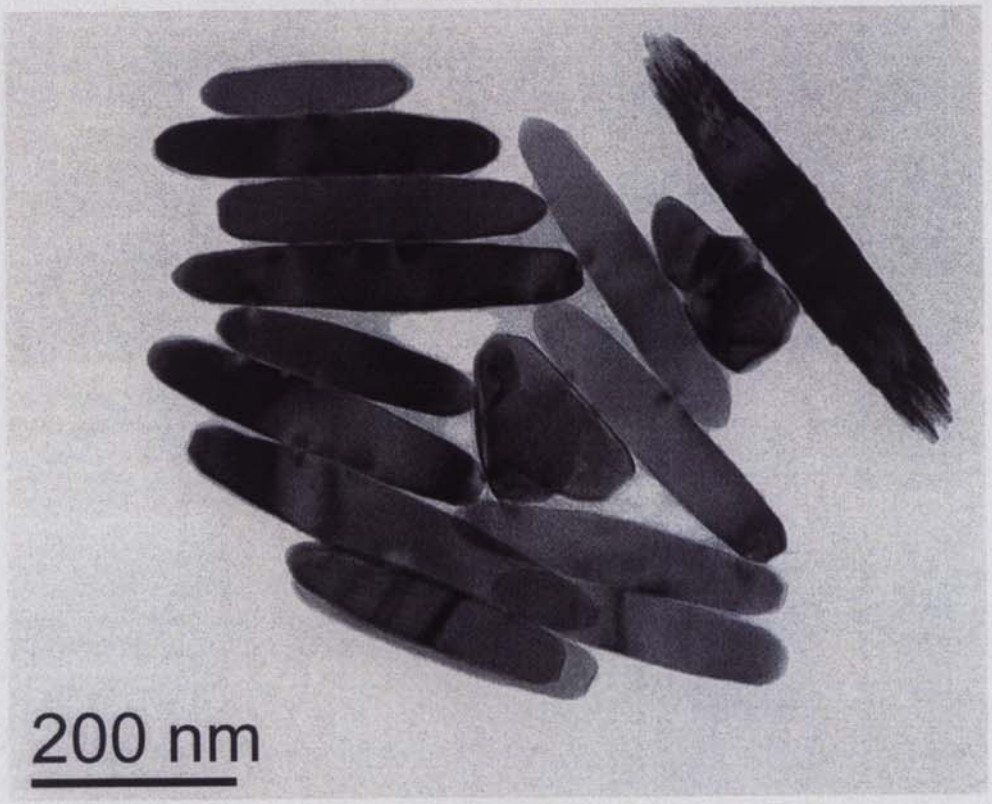
TiO_2 Nano Dots

TiO_2 Dots ("RHT-137")

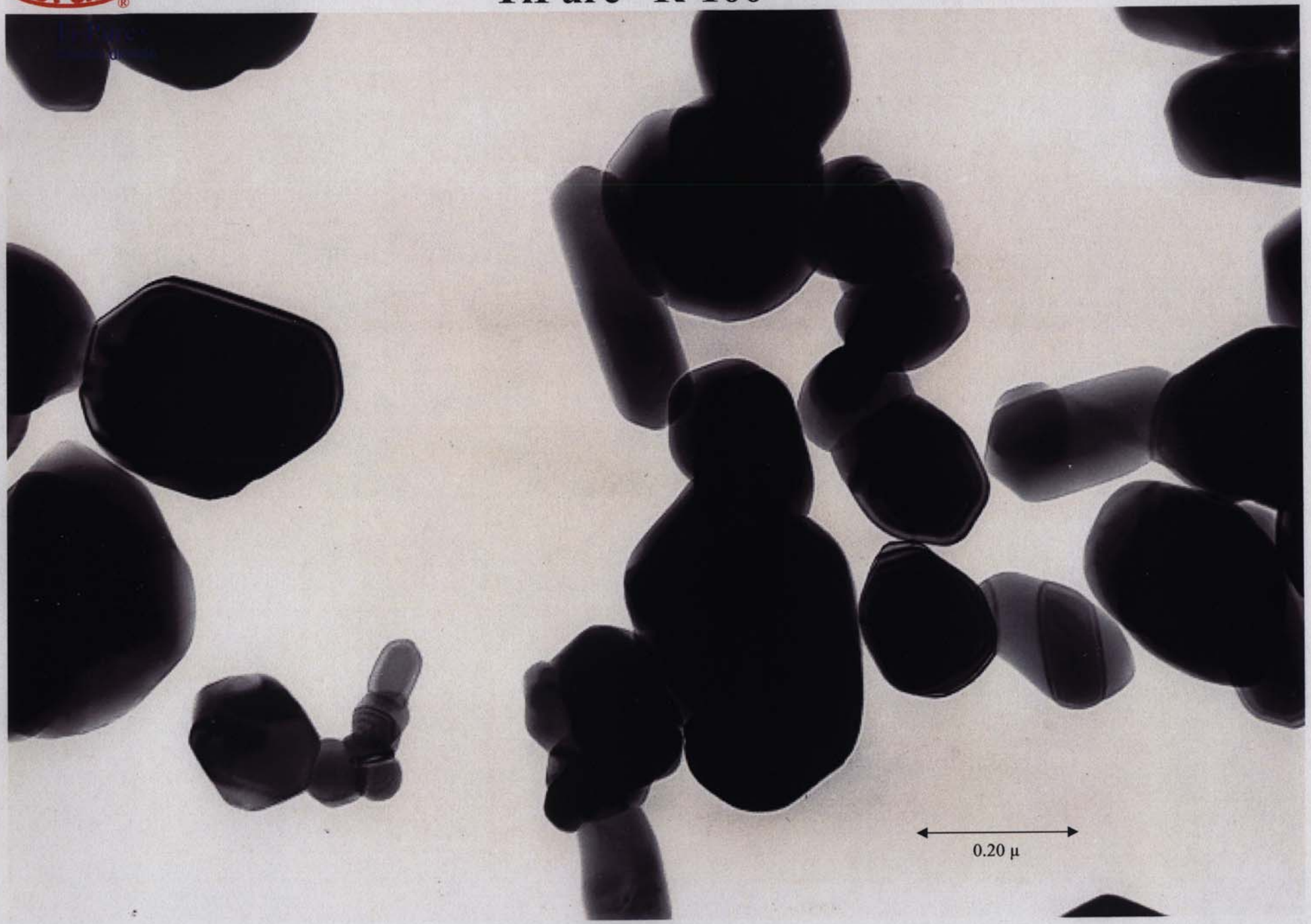


TiO_2 Nano Rods

TiO_2 Rods ("Chem_EE")



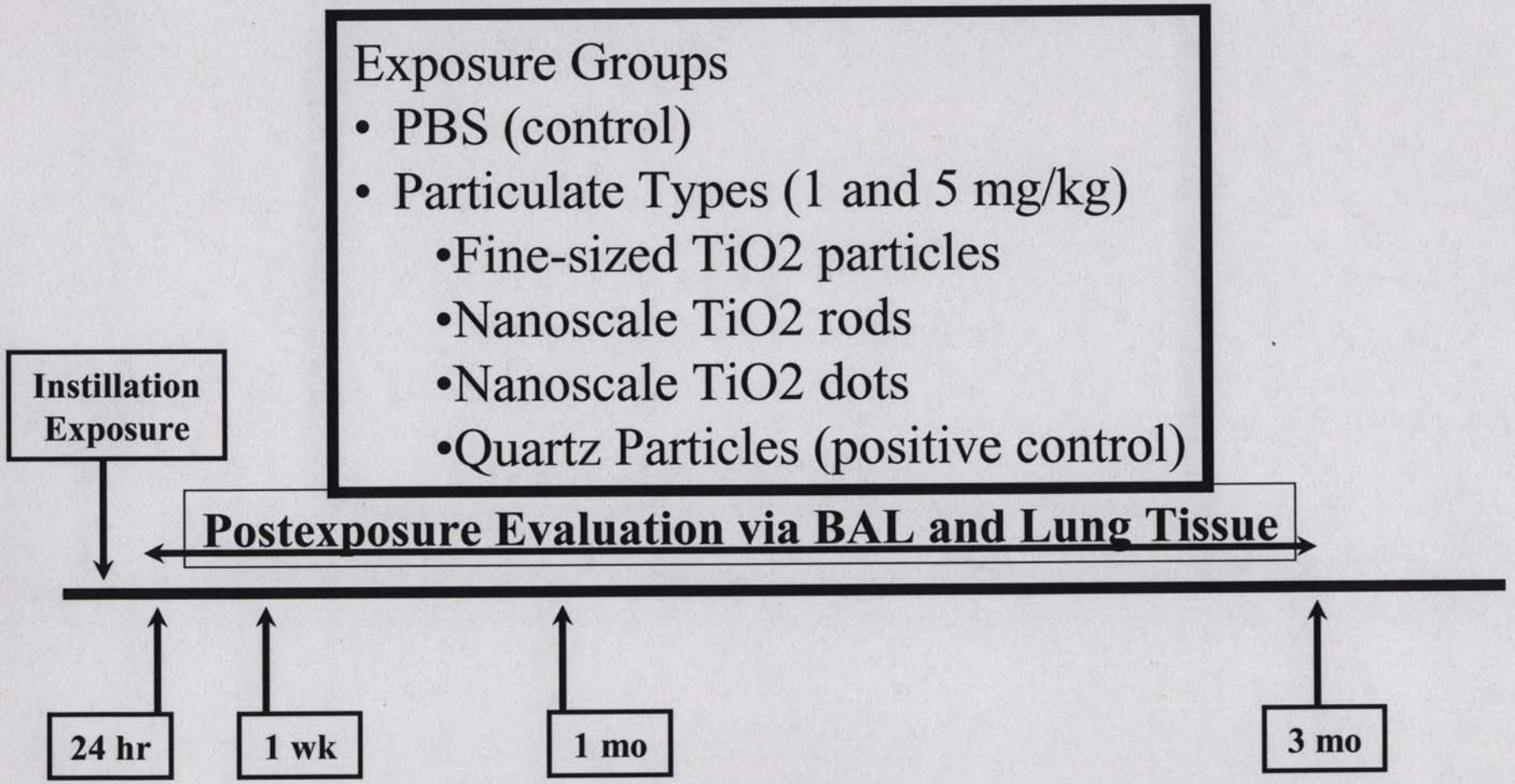
TiPure® R-100



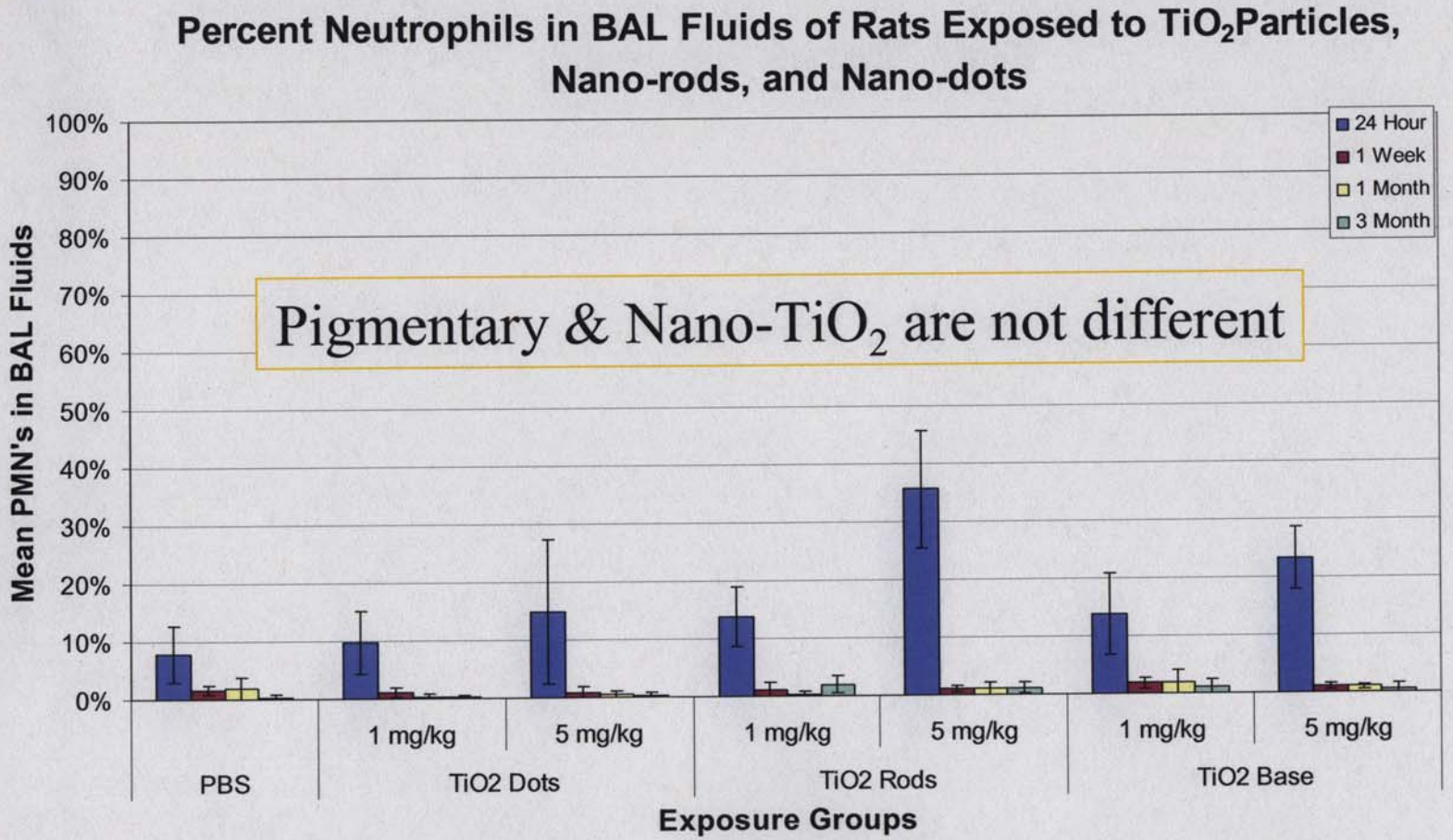
Characterization of Nanoscale TiO_2 and Quartz Particles

	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**

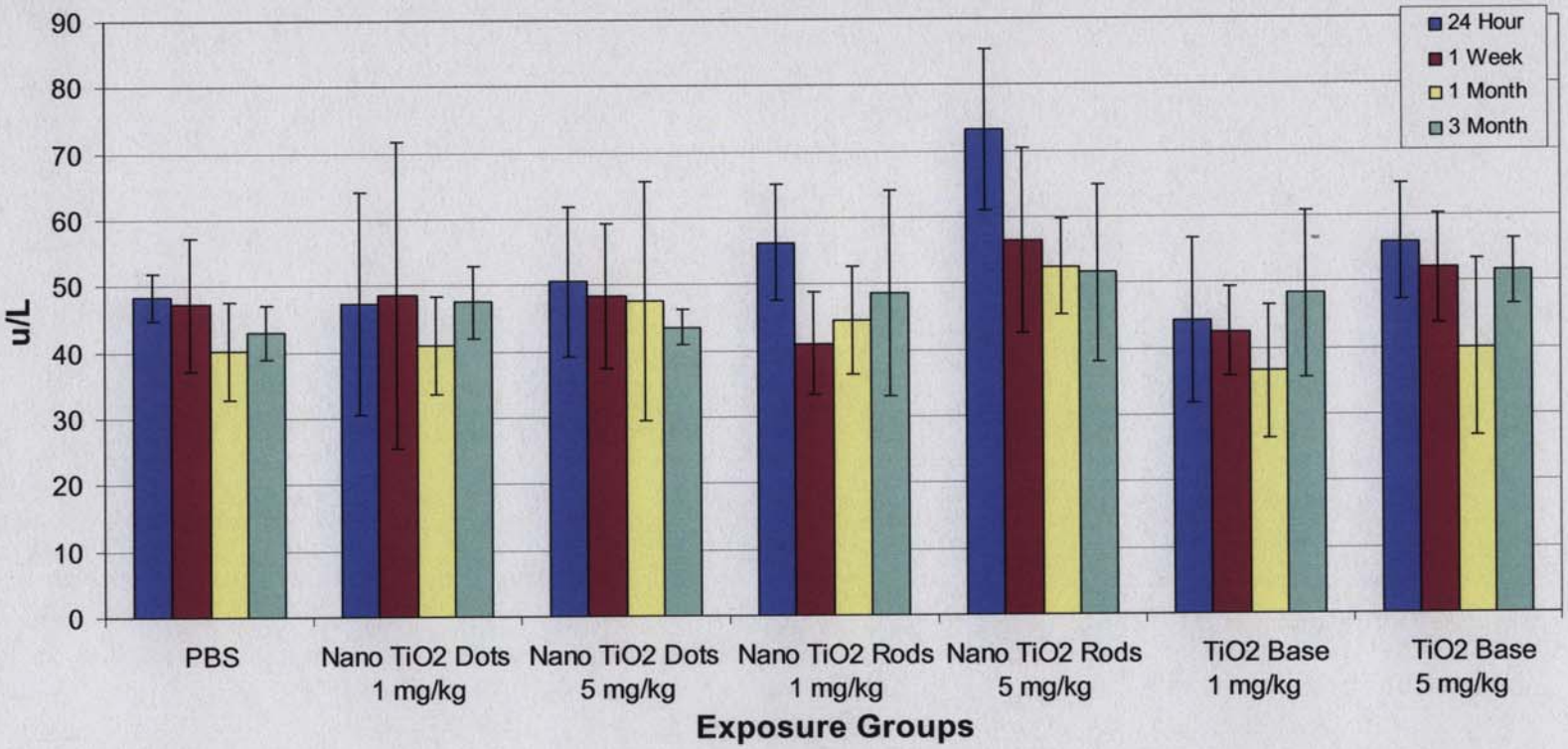
Protocol for Nanoscale TiO_2 Pulmonary Bioassay Study



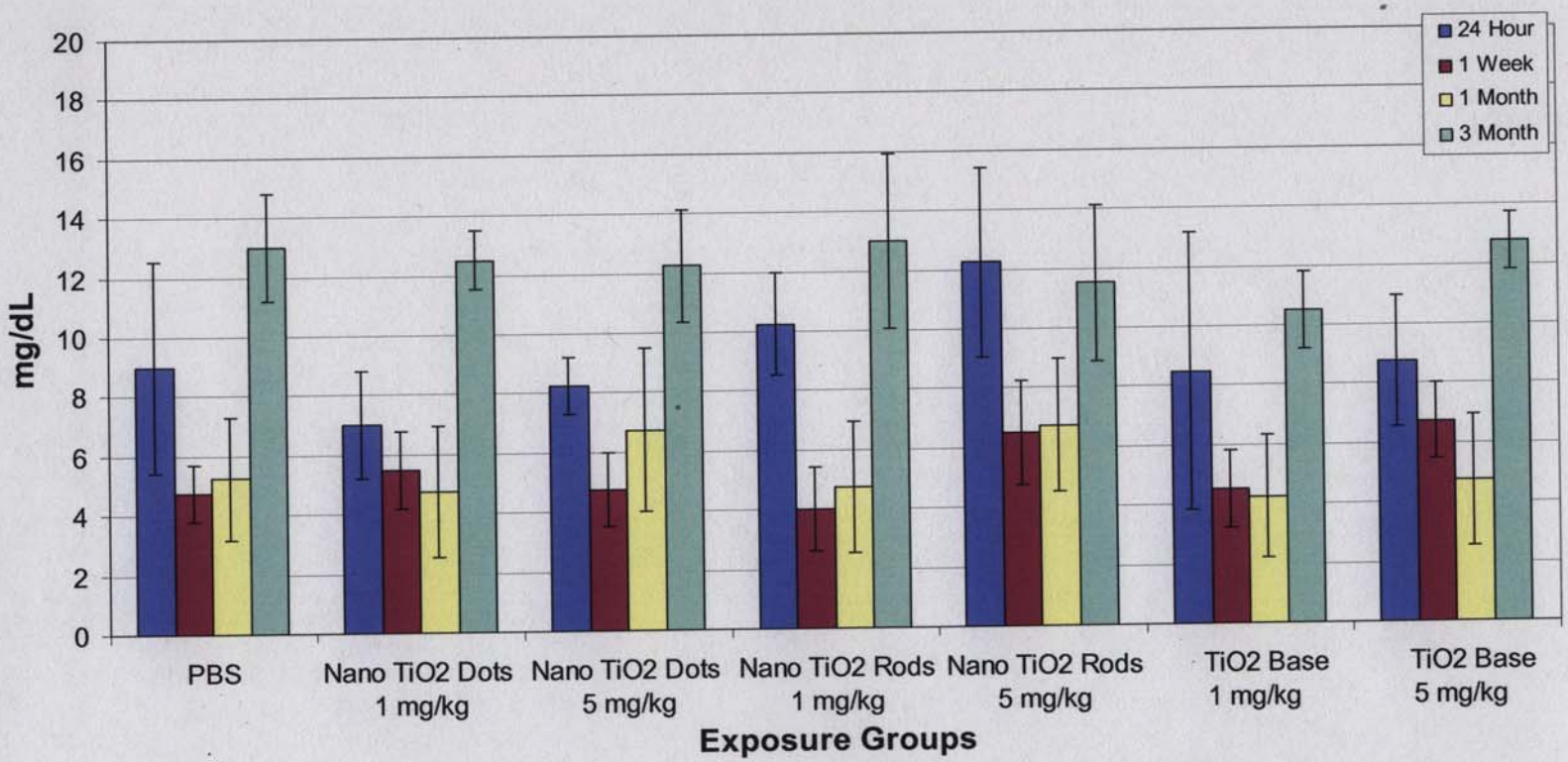
Collaborative Studies with Rice University: TiO_2

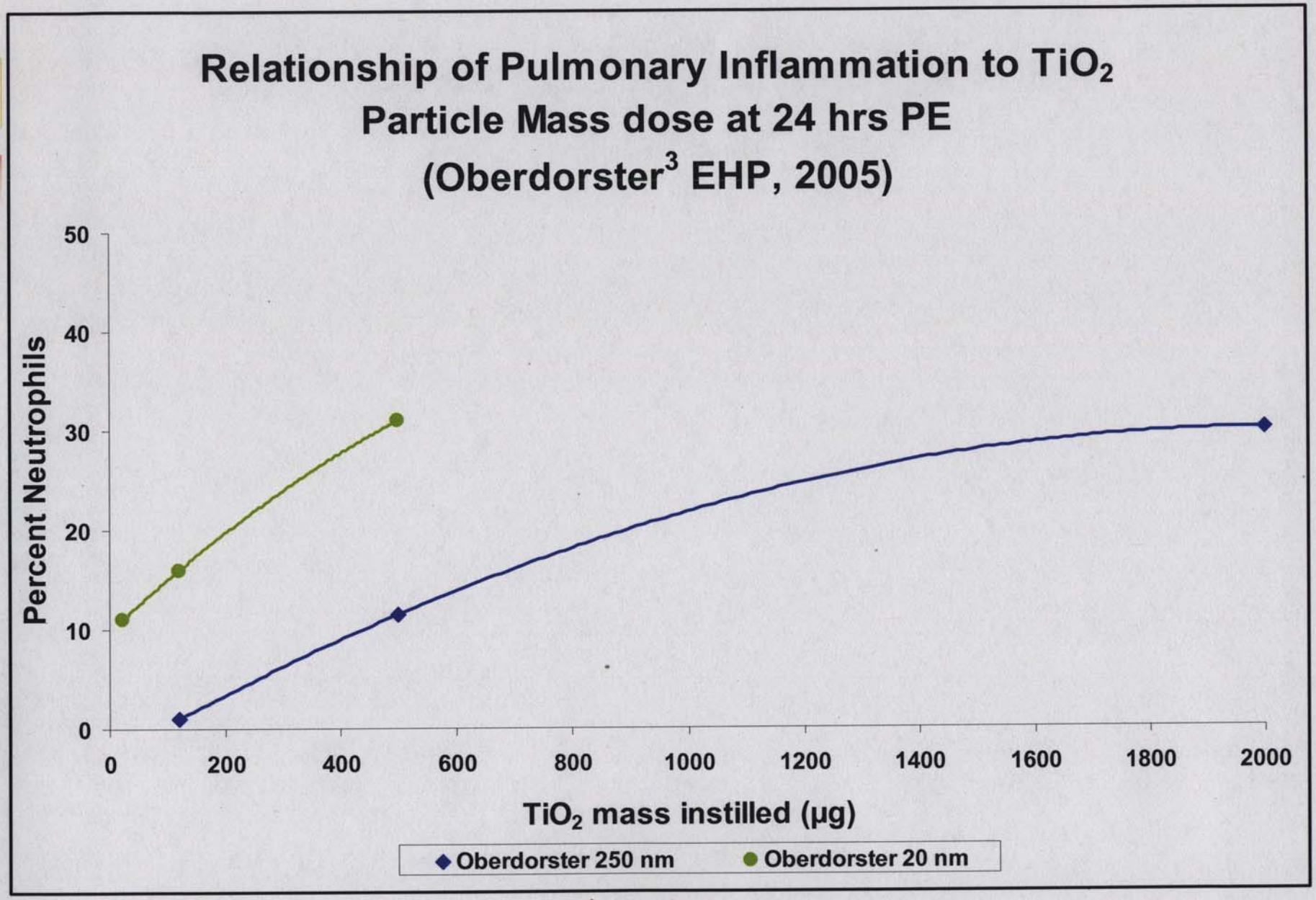


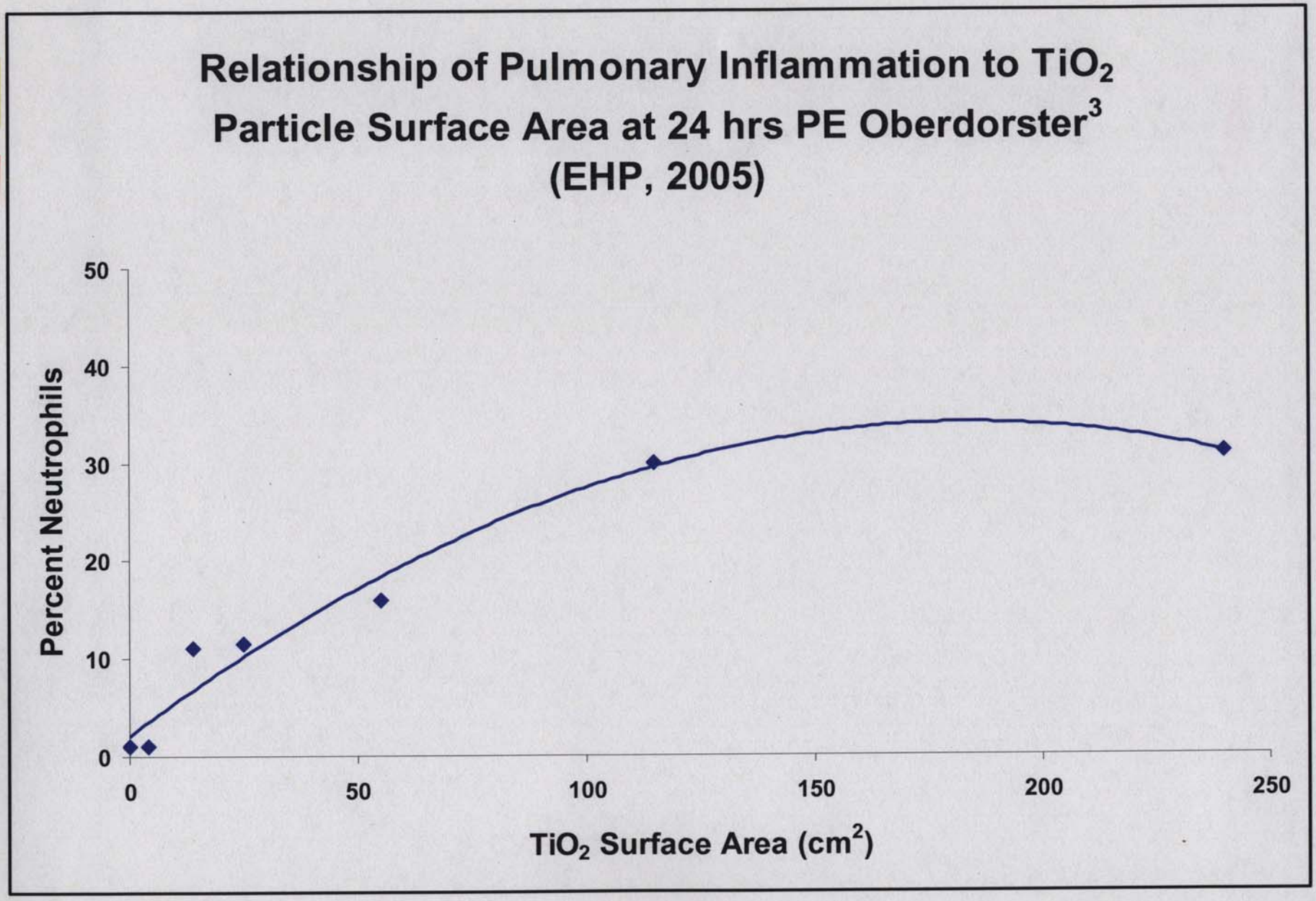
BAL Fluid LDH Values In Rats Exposed to TiO_2 Particles, Nano-rods, and Nano-dots

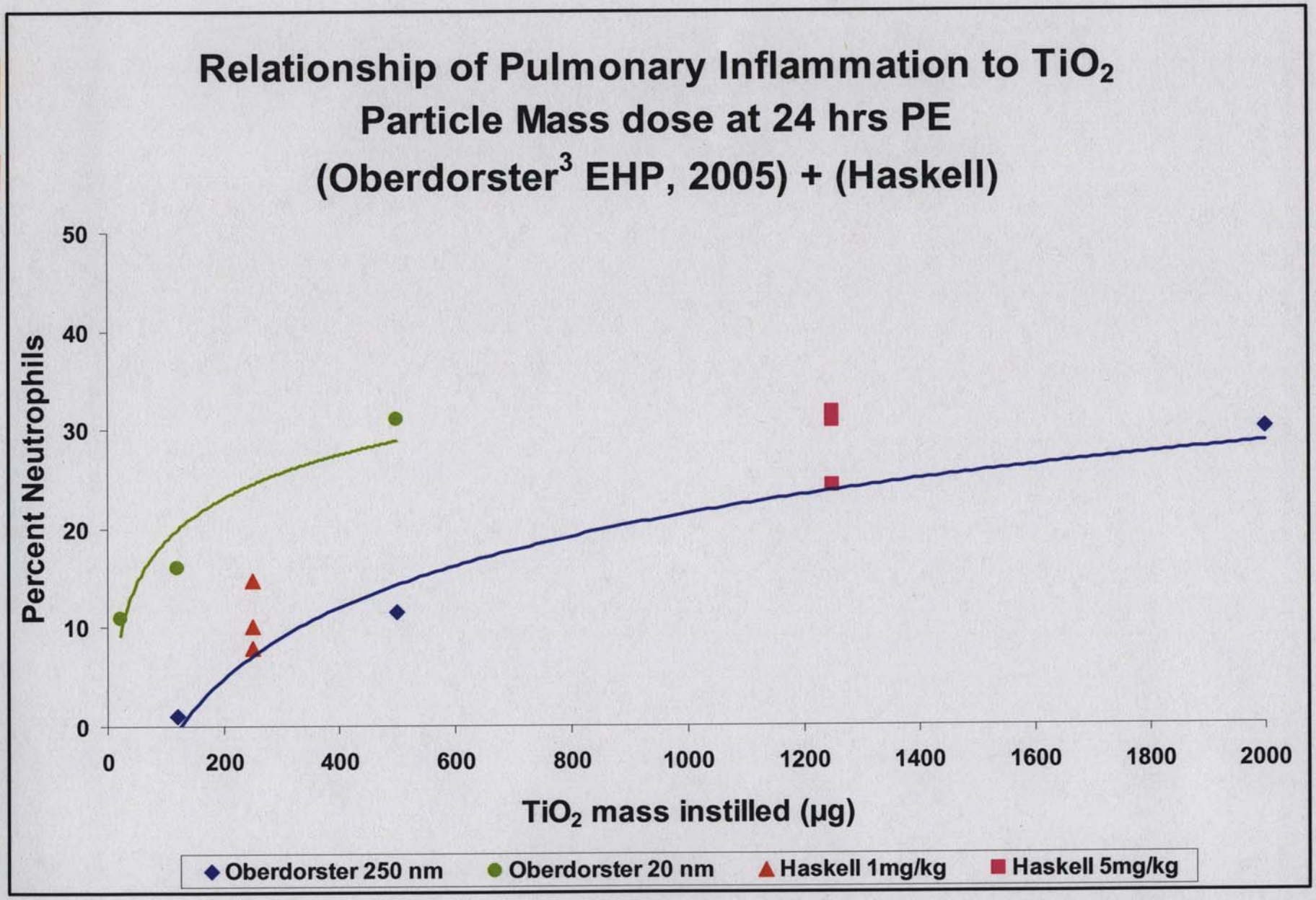


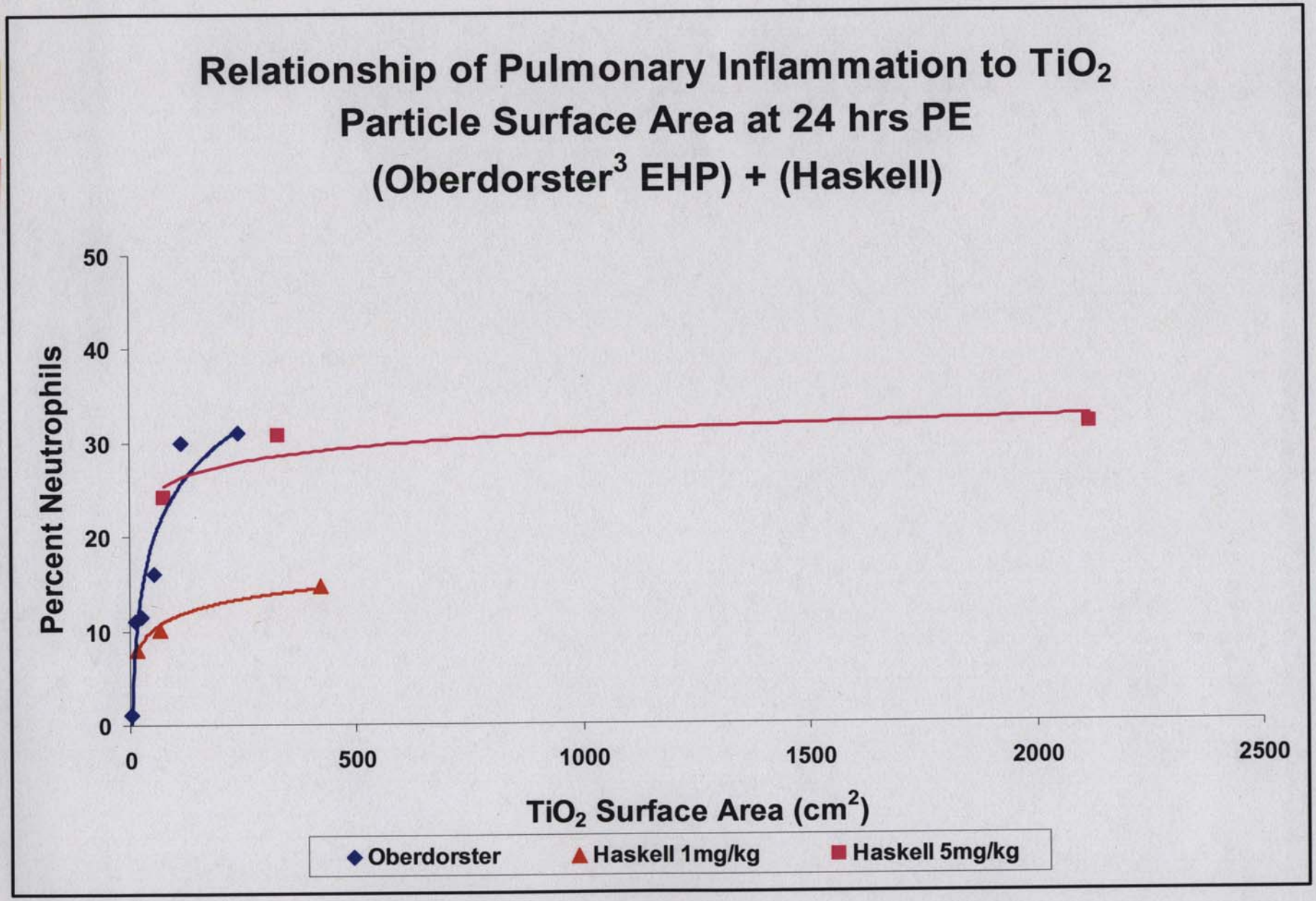
BAL Fluid MTP Values In Rats Exposed to TiO_2 Particles, Nano-rods, and Nano-dots




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


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)


Relationship of Pulmonary Inflammation to TiO_2
Particle Mass dose at 24 hrs PE
(Oberdorster[3] EHP, 2005) + (Haskell)
Percent Neutrophils
50
40
30
20
10
0
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


Relationship of Pulmonary Inflammation to TiO_2
Particle Surface Area at 24 hrs PE
(Oberdorster[3] EHP) + (Haskell)
Percent Neutrophils
50
40
30
20
10
0
0
500
1000
1500
2000
2500
TiO_2 Surface Area (cm^2)
Oberdorster
Haskell 1mg/kg
Haskell 5mg/kg

Summary of Results and Significance

- These results provide the first example of nanoscale particle-types which are not more cytotoxic or inflammogenic to the lung compared to larger-sized particles of similar composition. Furthermore, these findings run counter to the postulation that surface area is a major factor associated with the pulmonary toxicity of nanoscale particle-types.

Summary of Results and Significance relative to TiO_2 CIB

- The results of this study and other hazard studies demonstrate that the P25 ultrafine TiO_2 particles utilized in the Heinrich et al., and Bermudez et al. studies are not representative for all ultrafine or Nano TiO_2 particle-types. Therefore the recommendation of an exposure limit of 0.1 mg/m^3 becomes problematic.

Summary Conclusions - I

- General document and pertinent literature review is for the most part, comprehensive
- However, many of the summaries are not properly interpreted.
- The authors seem to de-emphasize the critically relevant studies by Nikula et al., and Bermudez et al.
- The authors draw the erroneous conclusion that since humans have slow dust clearance response, leading to particle overload, the human response might be similar to the rat response, possibly leading to lung tumors. Thus the corresponding risk assessment models are inappropriate.

Summary Conclusions - II

- Rats are uniquely sensitive to developing lung responses to poorly soluble particulates at particle overload concentrations. This response does not occur in ANY other species.

Summary Conclusions - III

- Current Mode of Action Scenario for Development of Lung Tumors in Rats exposed to Overload concentrations of PSPs

- In rats→ chronic exposure to TiO2 → Particle Overload → sustained inflammation → epithelial cell proliferation, septal fibrosis and fibroproliferative effects → metaplasia → mutations → lung tumors

- In humans → chronic exposure to PSP → Particle Overload → low degree of inflammation → little or no Fibrosis → no Mutations → No lung tumors

Summary Conclusions - IV

- Poor Justification for the suggested RELs for pigment grade (1.5 mg/m^3) or ultrafine TiO_2 particles (0.1 mg/m^3) (John Tomenson)

- Throughout the CIB document, the authors fail to adequately characterize the crystal structures and particle sizes (physical characteristics) used by the investigators in the various studies.

Summary Conclusions - V

- Epidemiology results from 4 extensive studies are negative for lung cancer in TiO_2 workers.
- Ultrafine TiO_2 particles are comprised of different crystal structures (anatase or rutile) and different particle sizes and the suggestion that P25 is representative for all ultrafine TiO_2 particle-types is inappropriate.
- Acknowledgment by other federal agencies and commissions that TiO2 is a low toxicity dust and therefore has been de-listed by USEPA from their TRI listing.