Health Effects of Inhaled Engineered and Incidental Nanoparticles

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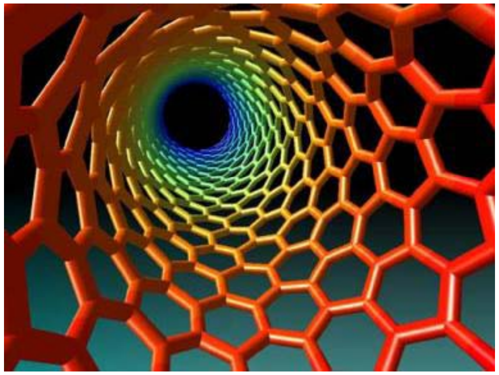
Overview

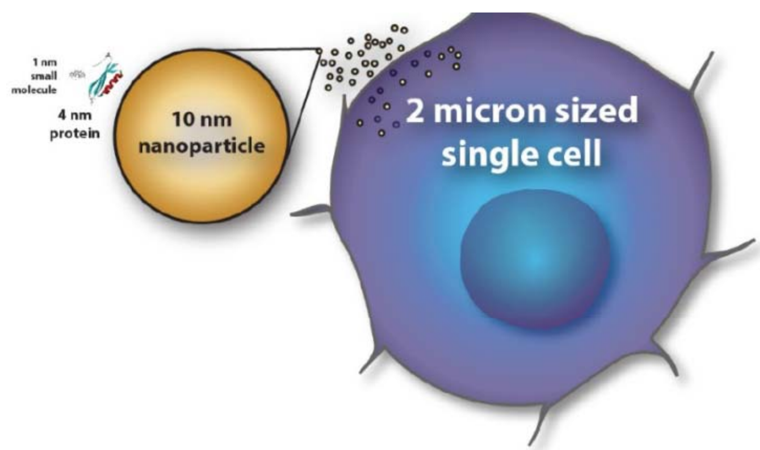


- Emphasize importance of understanding physical and chemical characteristics of nanomaterials
- Consider past experience as guidance for identifying important properties of nanomaterials
- Review past research in regards to what is known about size, shape, surface chemistry, etc. and describe what this means for understanding the hazards of engineered nanomaterials

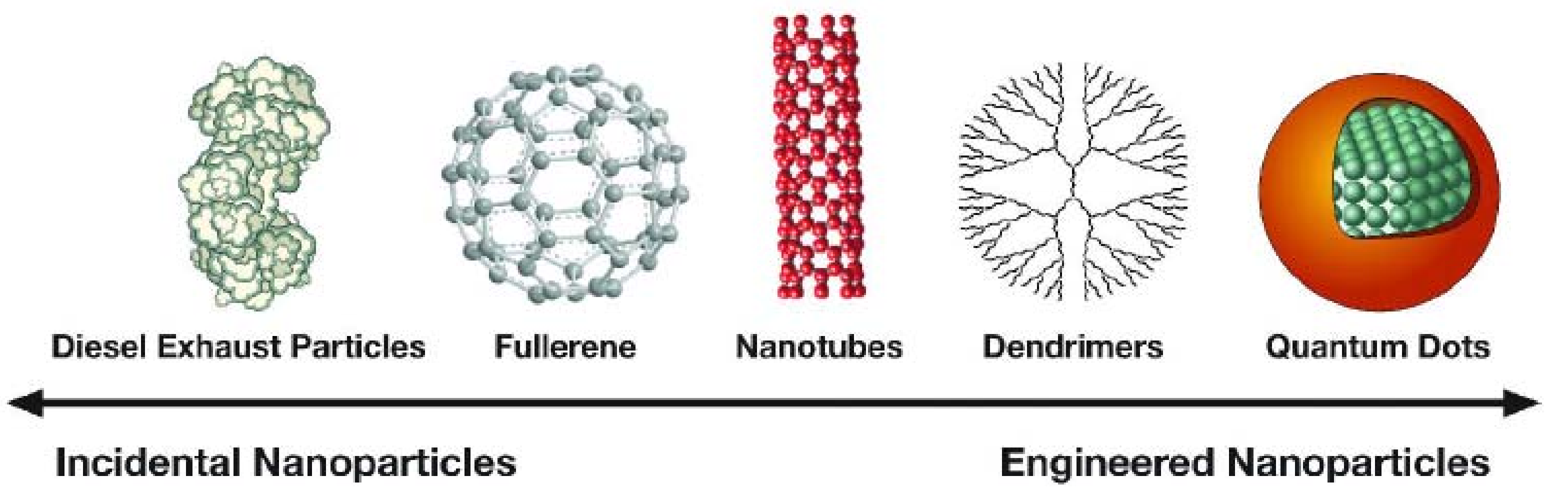
www.chemistryland.com/.../NanoTube500.jpg (photo source)

Nanotechnology

- Field that "involves a wide range of technologies that measure, manipulate, or incorporate materials and/or features with at least one dimension between approximately 1 and 100 nanometers (nm). Such applications exploit the properties, distinct from bulk/macroscopic systems, of nanoscale components."



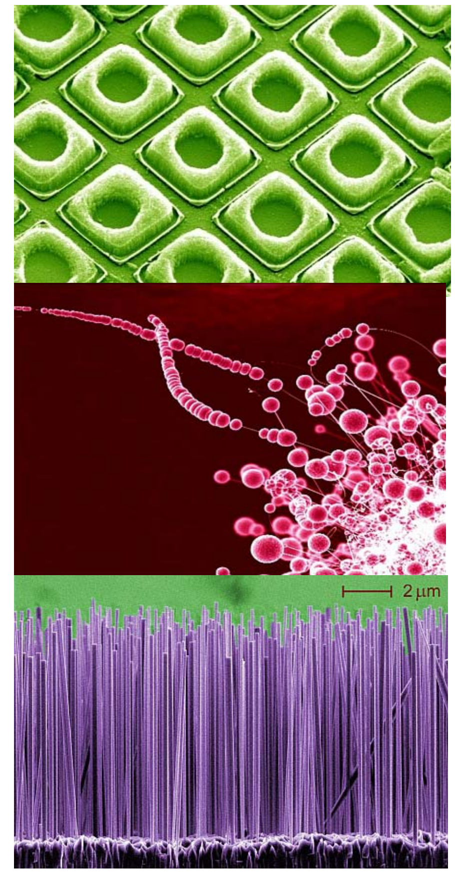
Incidental v. Engineered Nanoparticles



Stern and McNeil 2007

Different Types of Nanomaterials

- Carbon nanotubes
- Silver nanoparticles
- Metal oxides
- Nanocrystals
- Nanowires
- Superparamagnetic iron oxide
- Ligand-coated gold
- Dendrimers
- Quantum dots
- Micelles
- Liposomes
- Polymers



Nanoscience Images
by Zhengwei Pan

http://www.itmweb.com/bimages/nanowire2.jpg; http://www.cnms.ornl.gov/nanosci/lp5.shtm (photo source)

Applications and Potential Sources of Exposure

Biomedical

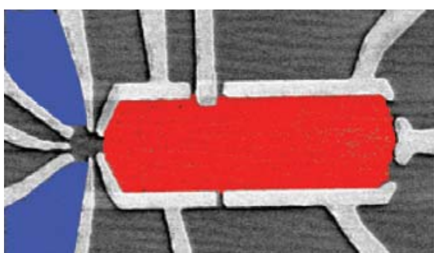
- Drug carriers
- Tumor imaging & treatment
- Cell-targeted therapy
- Cell sensors & microchips
- Cell and tissue scaffolds

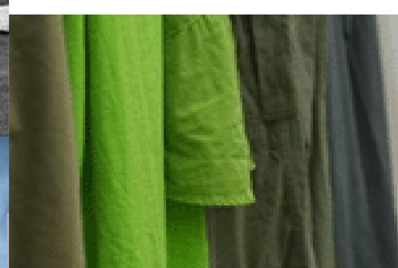
Industrial

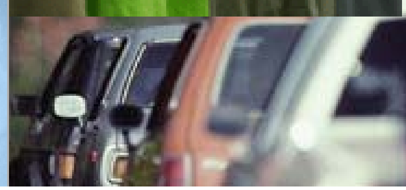
- Molecular switches
- Battery electrodes
- Solar & fuel cells
- Composites
- Semiconductor
- Remediation

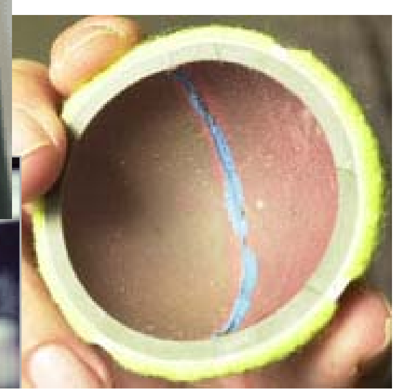
Consumer

- Appliances
- Food and beverage
- Textiles
- Filtration
- Sports equipment
- Electronics & computers
- Cosmetics
- Personal care products
- Automotive equipment

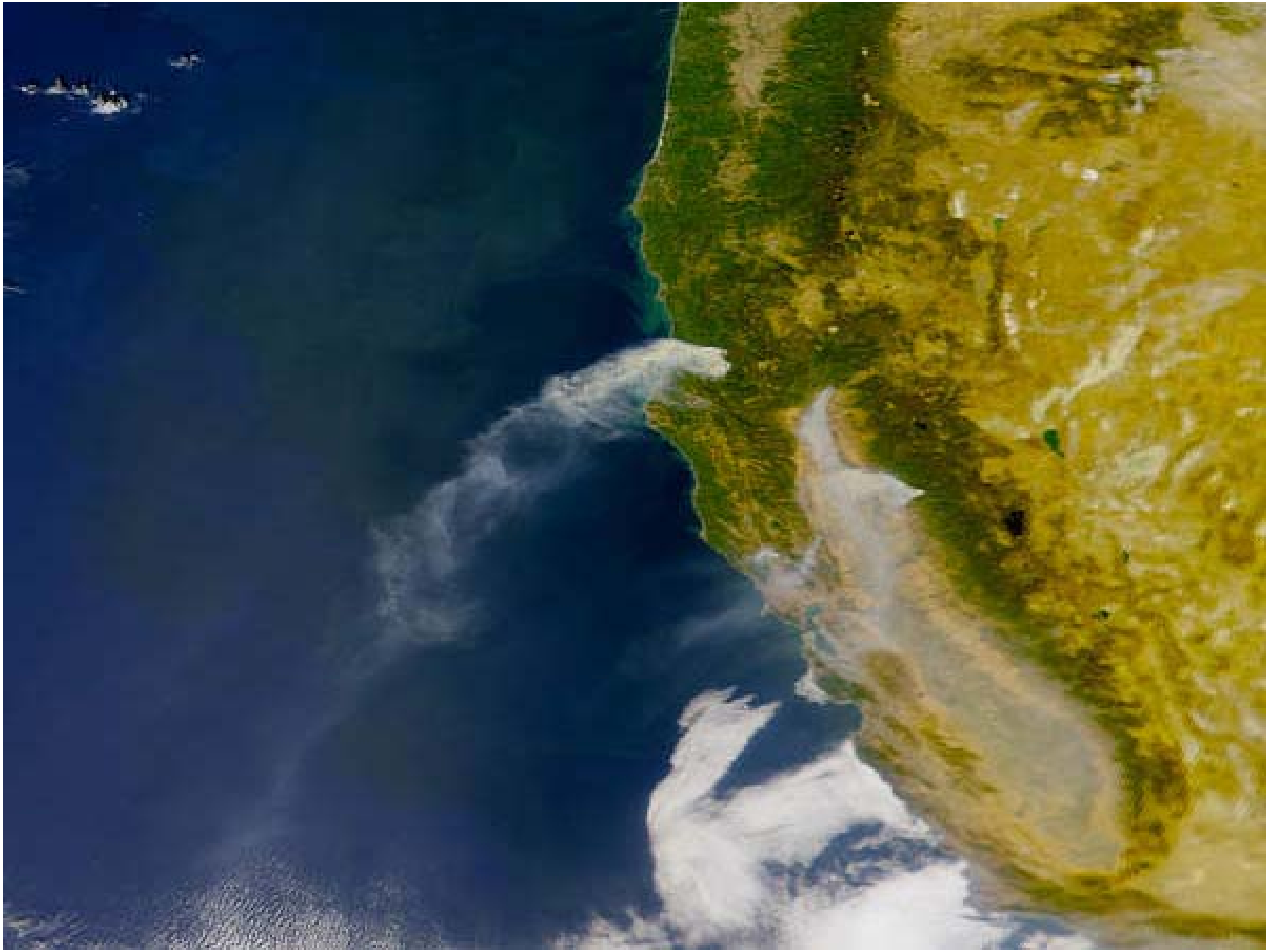




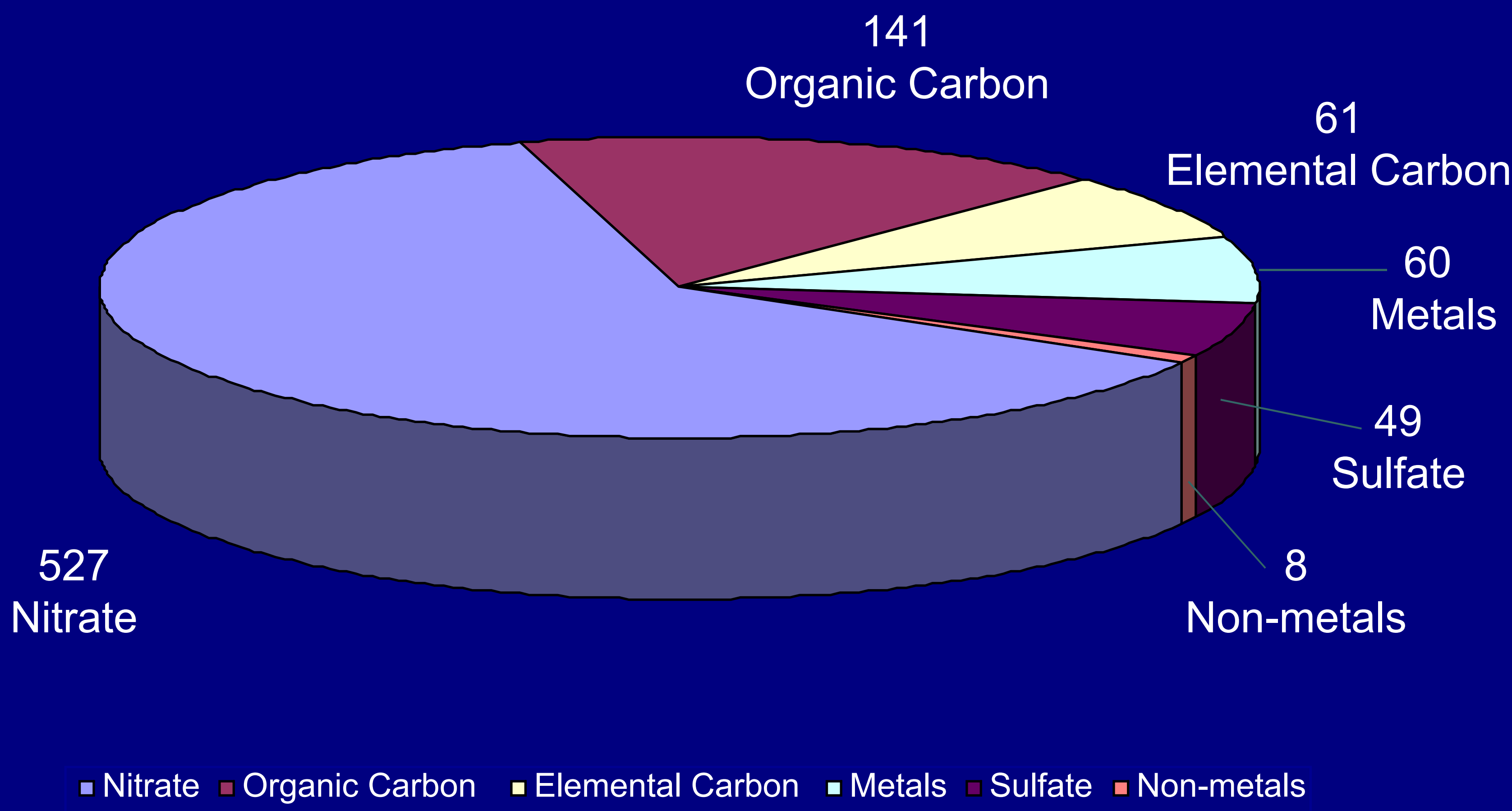




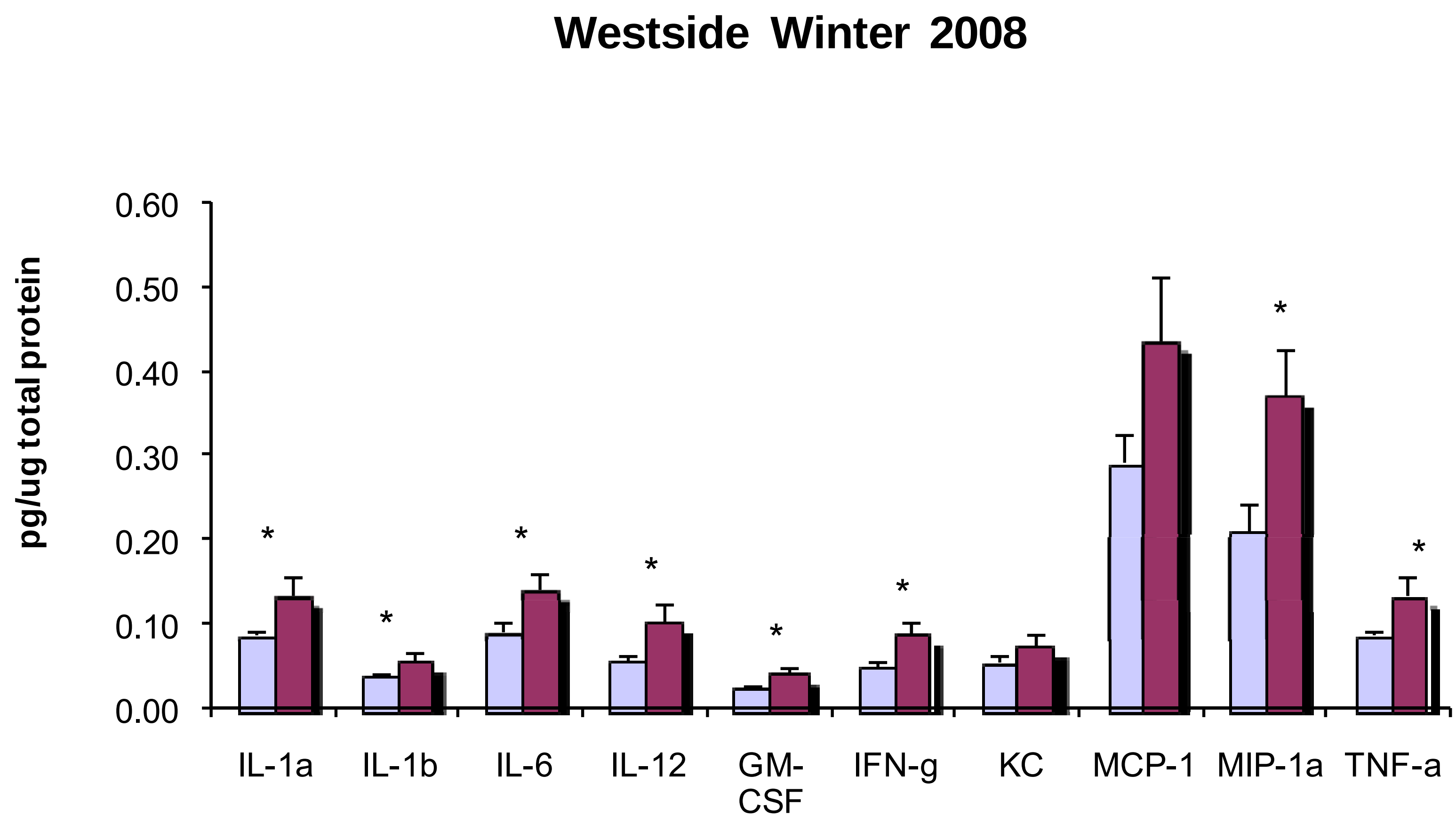
Experience with Incidental Nanoparticles can Provide Insights into the Possible Hazards of Engineered Nanoparticles



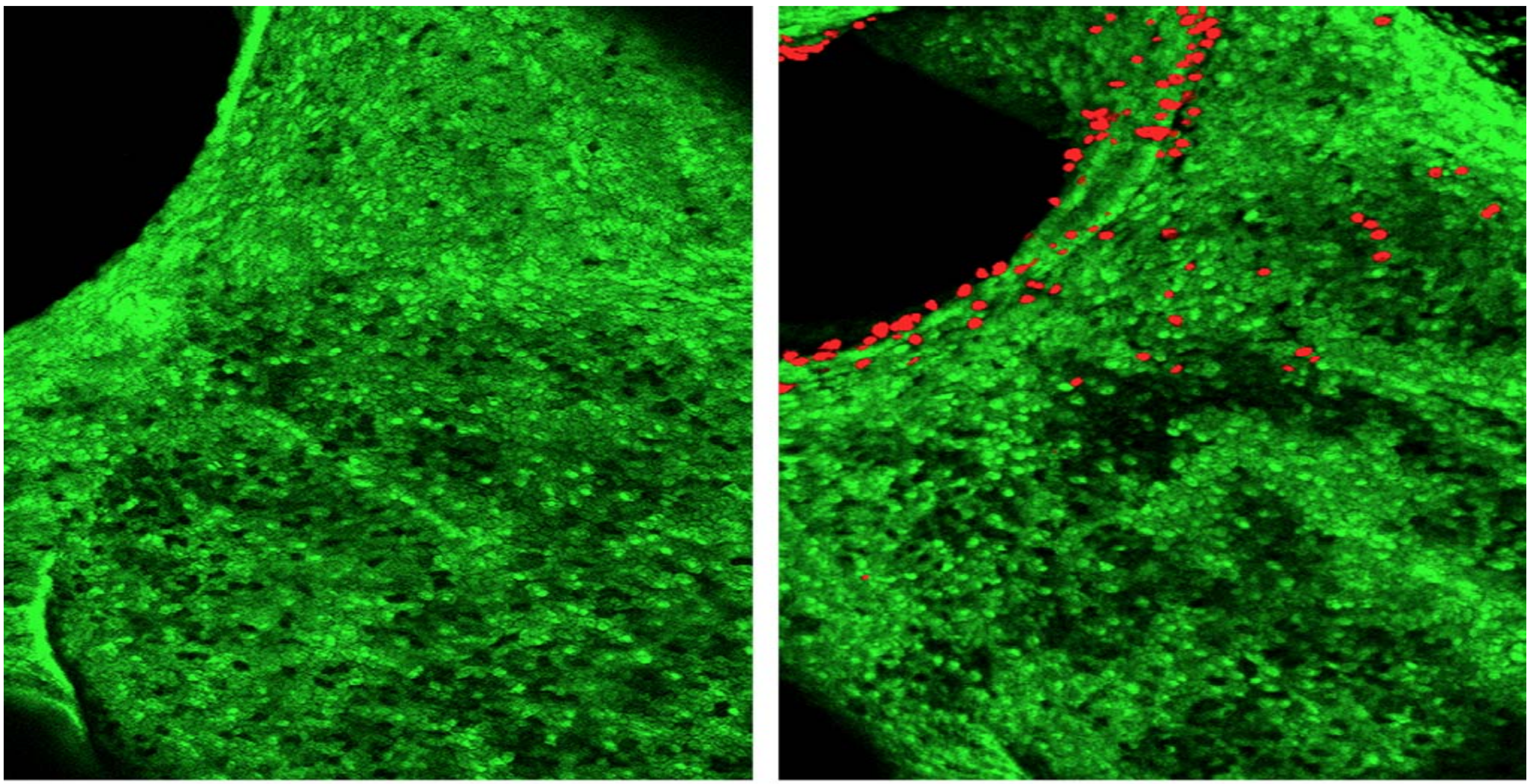
Chemical Composition of San Joaquin Valley Fine Aerosol



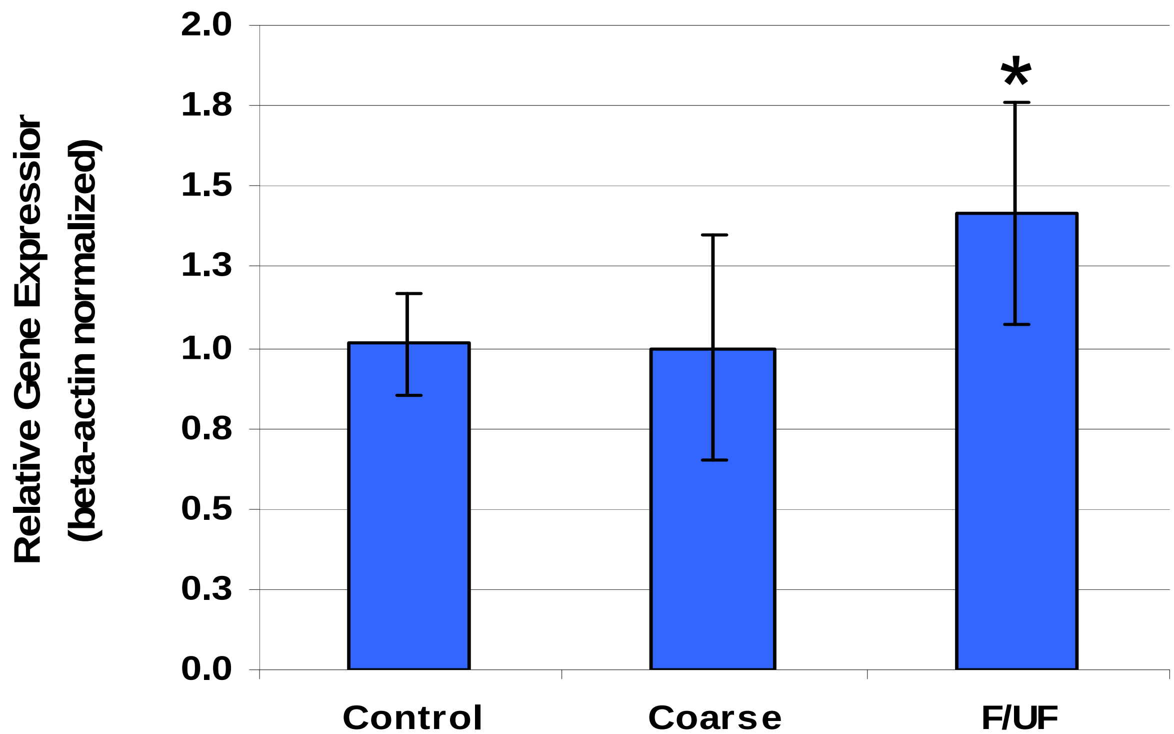
Lung Cytokine/Chemokines from Exposure to Concentrated Ambient Particles (CAPs)



Epithelial Cytotoxicity of Airway Bifurcations following Particle Exposure



Interleukin-6 Expression in the Brain Following Exposure to Concentrated Ambient Particles (CAPs)



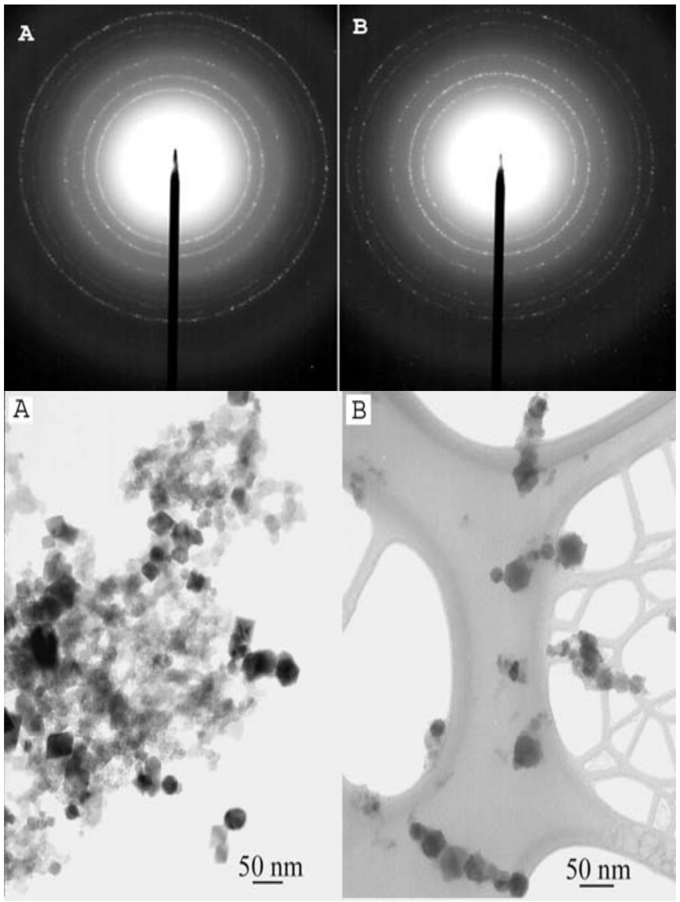
Real-time PCR was used to detect IL-6 expression levels * p = 0.024

Characteristics of Iron and Iron-Soot Particles

Iron-Soot

γ-Fe_2O_3
10-50 nm
particles

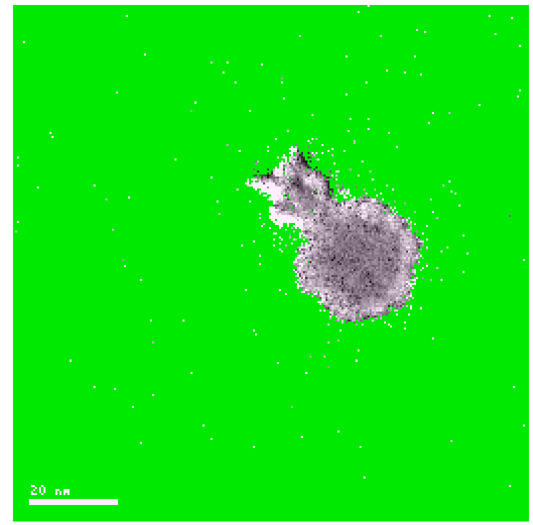
Up to 1 μm
agglomerates

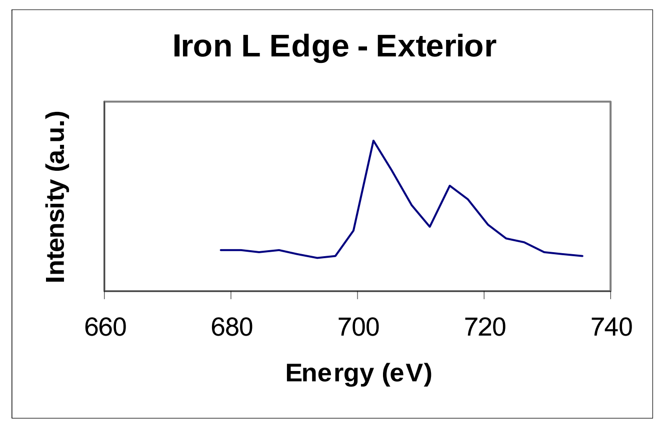


Iron Only

γ-Fe_2O_3
10-50 nm
particles

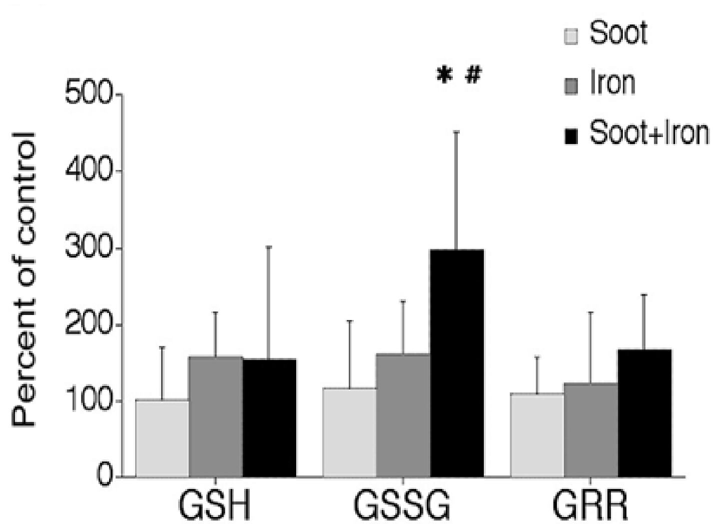
Up to 200 nm
agglomerates



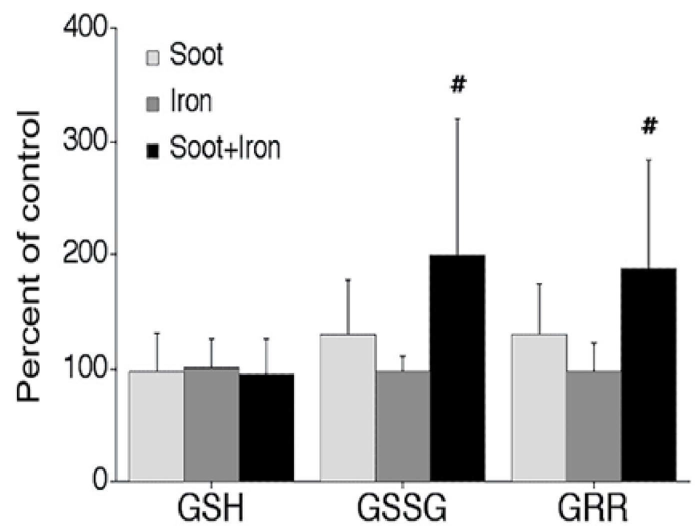


No change in phase or nanoparticle size

Oxidative Stress/Glutathione Levels in the Lungs of Animals Exposed to Iron Soot

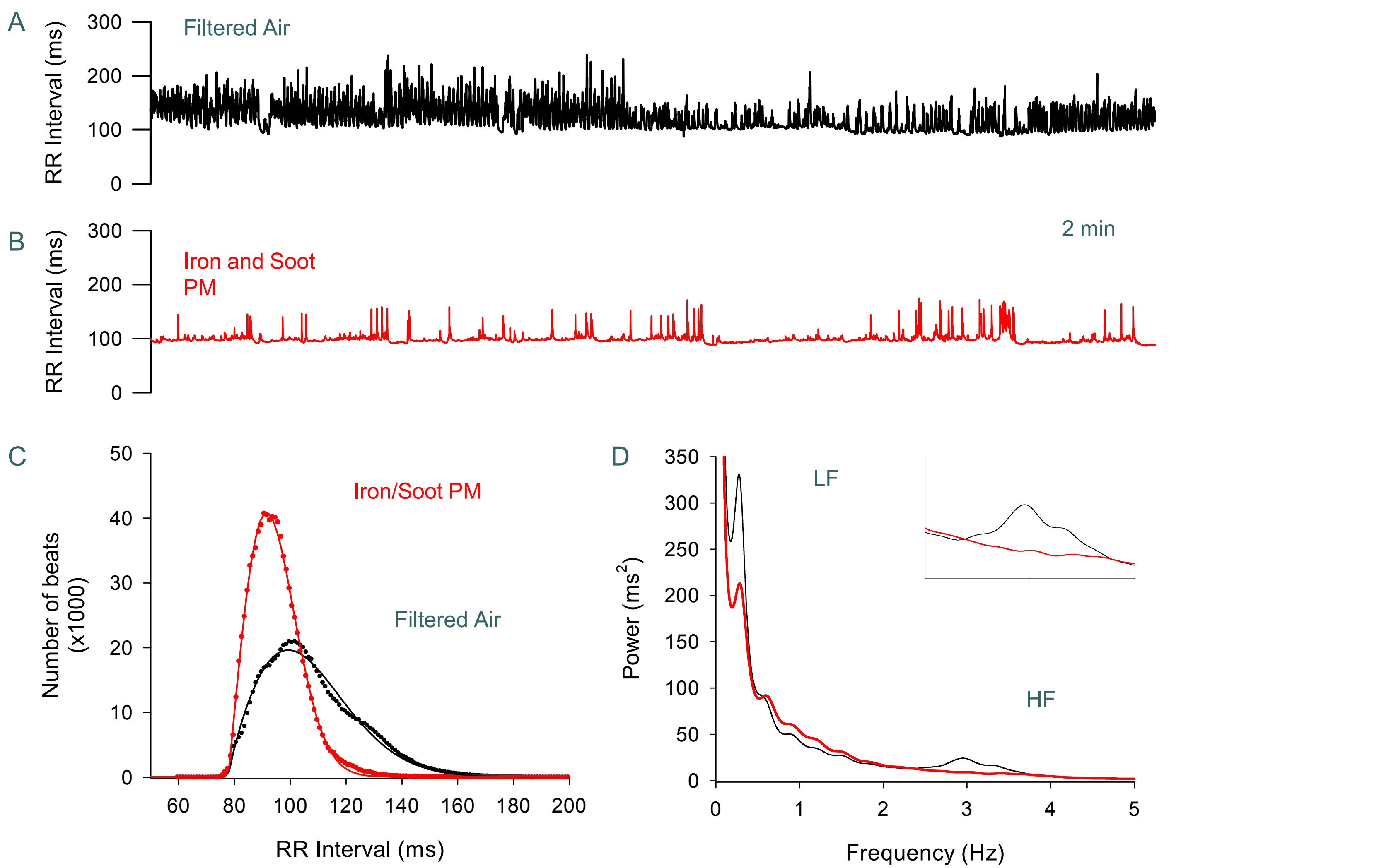


Bronchoalveolar Lavage



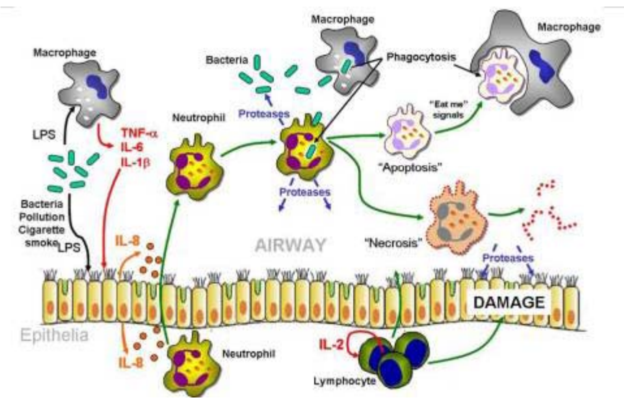
Lung Homogenate

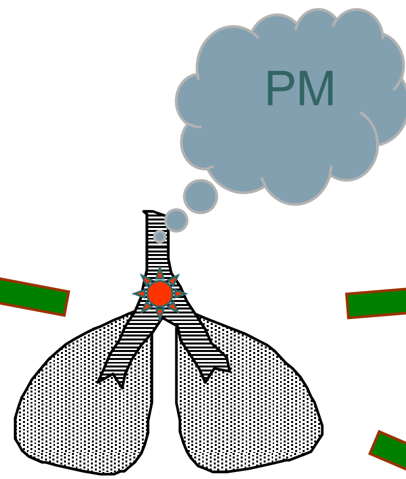
Reduced Heart Rate Variability Following Exposure to Iron Soot



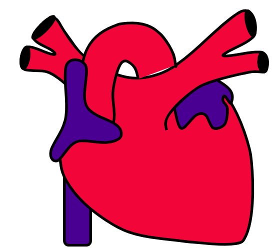
Potential Mechanisms

Inflammatory response

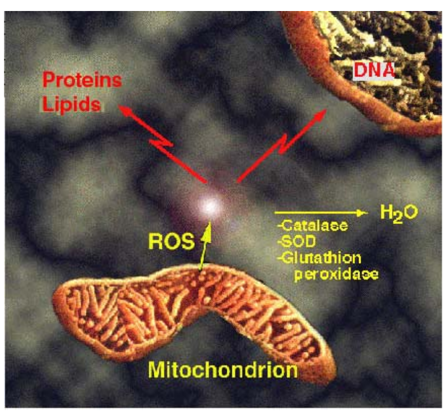




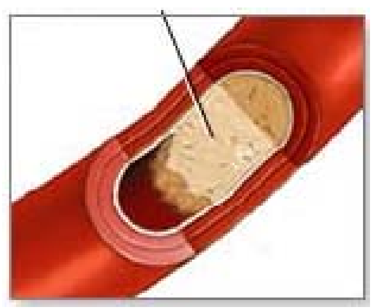
Decreased HRV



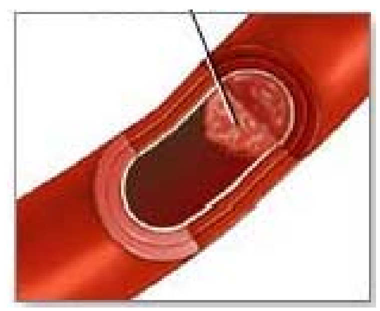
Endothelial dysfunction



Oxidative stress



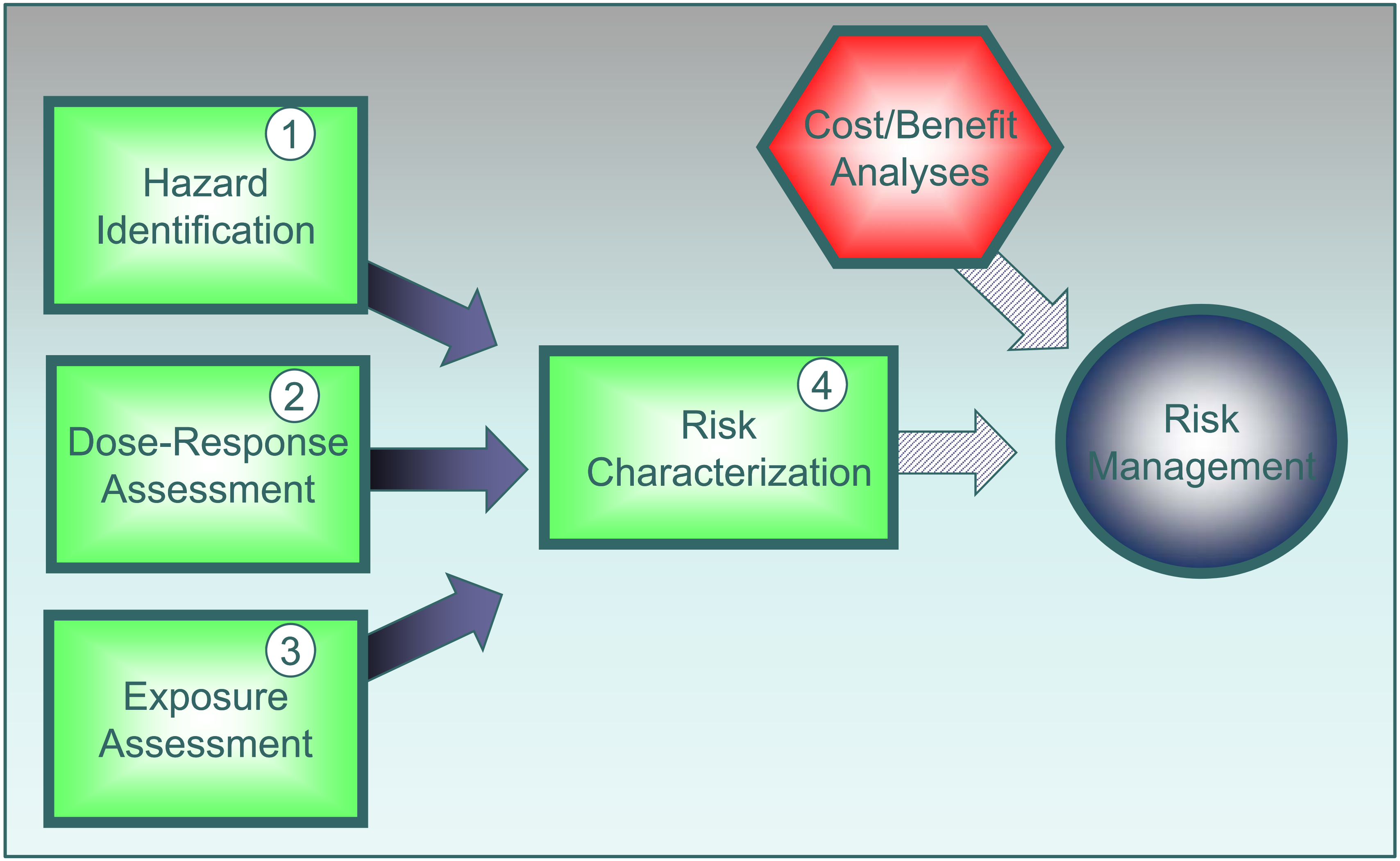
Atherosclerotic lesions



Platelet aggregation

What Physical and Chemical Characteristics of Engineered Nanoparticles Dictate Biocompatibility or Toxicity?

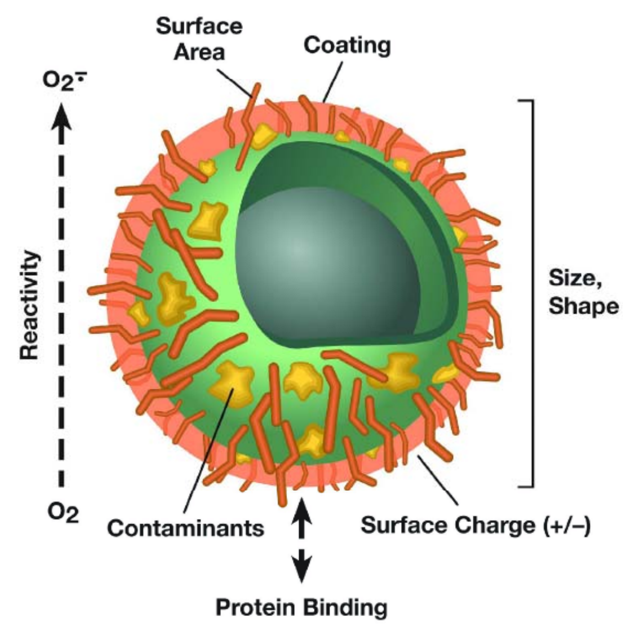
Placing Hazard Information in the Framework of Health Risk



Hazard Identification

- Chemical form
- Size
- Shape
- Surface area
- Number
- Density
- Mass
- Agglomeration
- Porosity
- Charge
- Reactivity

- Solubility
- Durability
- Crystalline structure
- Purity
- Antigenicity



Stern and McNeil 2007

Much Can Influence Hazard and Health Risk

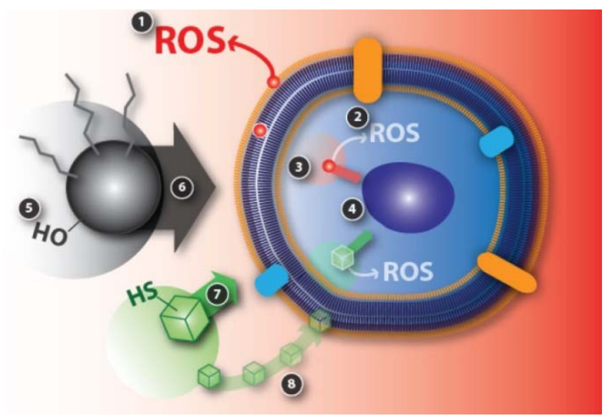
Exposure
Product form
Bulk concentration
Purity
Composite makeup
Friability
Stability/degradation
Method of handling
Exposure setting
Dispersion
Reentrainment
Bulk volume
Exposure frequency
Exposure duration

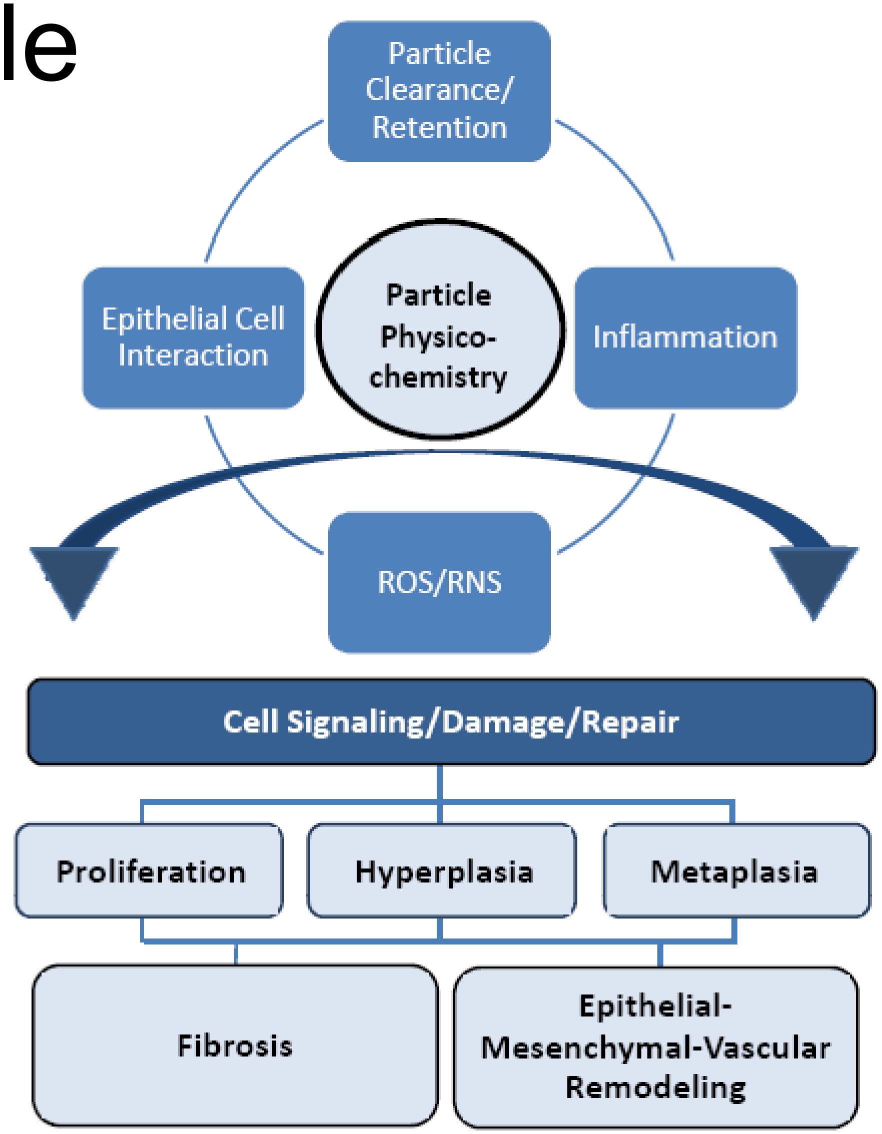
Particle Characteristics
Chemical form
Size
Shape
Surface area
Number
Density
Mass
Agglomeration
Porosity
Charge
Reactivity
Solubility
Durability
Crystalline structure
Purity
Antigenicity

Biological Fate
Exposure route
(ADME)
Absorption
Distribution
Metabolism
Elimination
Biopersistence

Biological Effects
Inflammation
Cytotoxicity
Oxidative stress
Fibrosis
Cellular and organ homeostasis
Genotoxicity

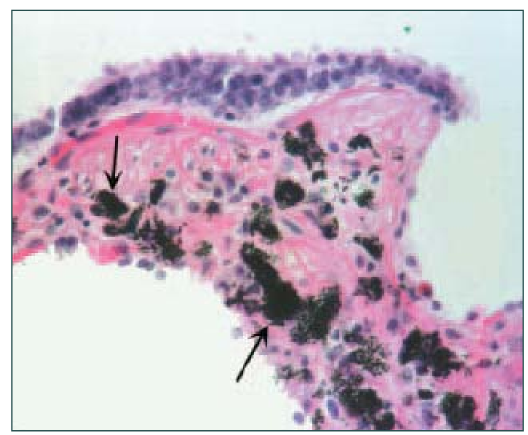
Various Possible Mechanisms of Toxicity





Markers for Toxicity

- Inflammatory cell profile
- Cytokine patterns
- Oxidative stress
  - Reactive oxygen species
  - Lipid peroxidation
  - Antioxidant capacity
- Structural/cellular remodeling
- Collagen
- Cell proliferation
- Apoptosis
- Physiologic impairment
- Cell cytotoxicity
- Immune cell function
- Metabolic capacity
- Secretory defense

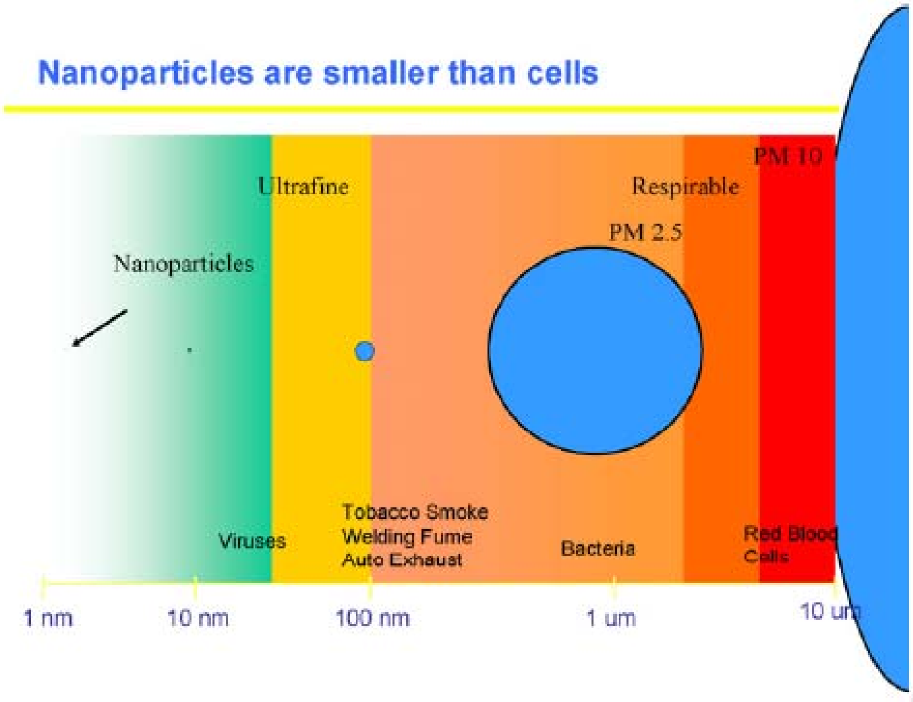


Pinkerton et al. 2000 EHP 108:1063–1069

Characteristics of Interest (OECD)

- Agglomeration/aggregation
- Water solubility
- Crystalline phase
- Dustiness
- Crystallite size
- Representative TEM picture(s)
- Particle size distribution
- Specific surface area
- Zeta potential (surface charge)
- Surface chemistry (where appropriate)
- Photocatalytic activity
- Pour density
- Porosity
- Octanol-water partition coefficient, where relevant
- Redox potential
- Radical formation potential

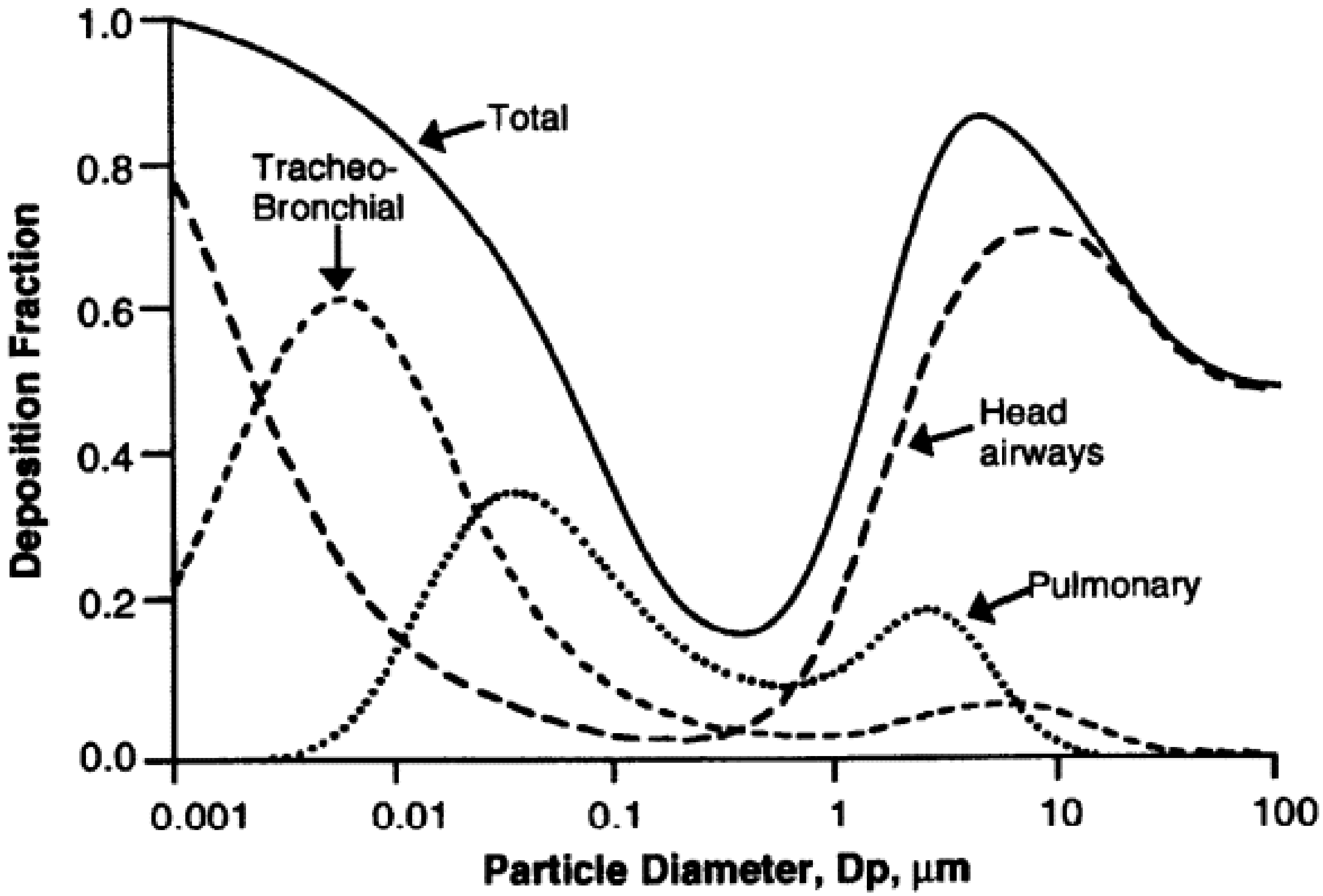
Particle Size



Considerations of Particle Size

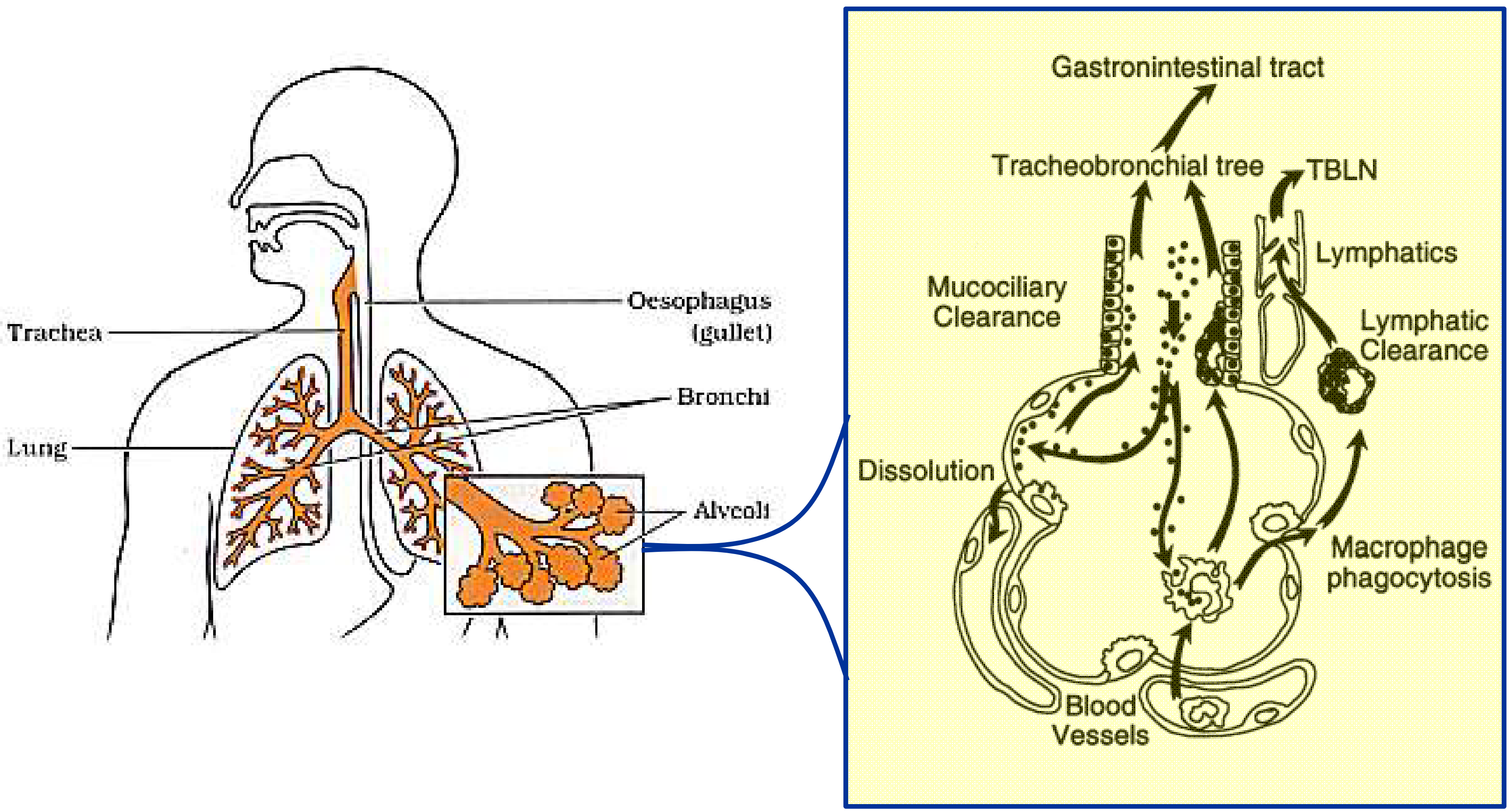
- There are many factors that can affect the size distribution of particles
  - Agglomeration
  - Suspension/media
  - Exposure route/method
  - Chemical bonds (e.g., van der Waals)
- This, in turn, can affect
  - Fate/transport
  - Retention/elimination
  - Cellular responses

Respiratory Deposition at Different Particle Sizes



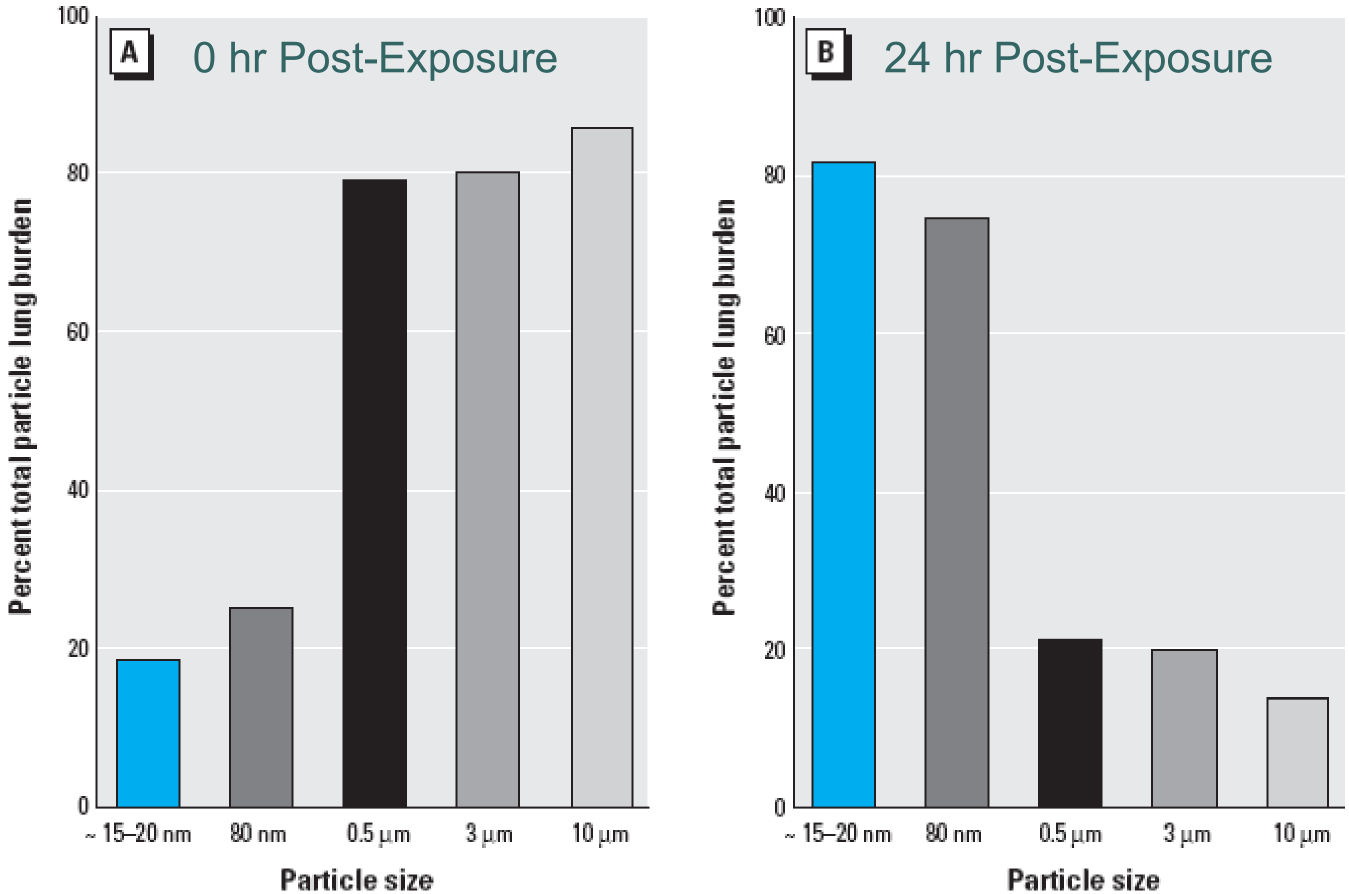
International Commission on Radiological Protection

Particle Fate and Transport



McClellen Comprehensive Toxicology

Nanoparticles are Retained Longer in the Lungs

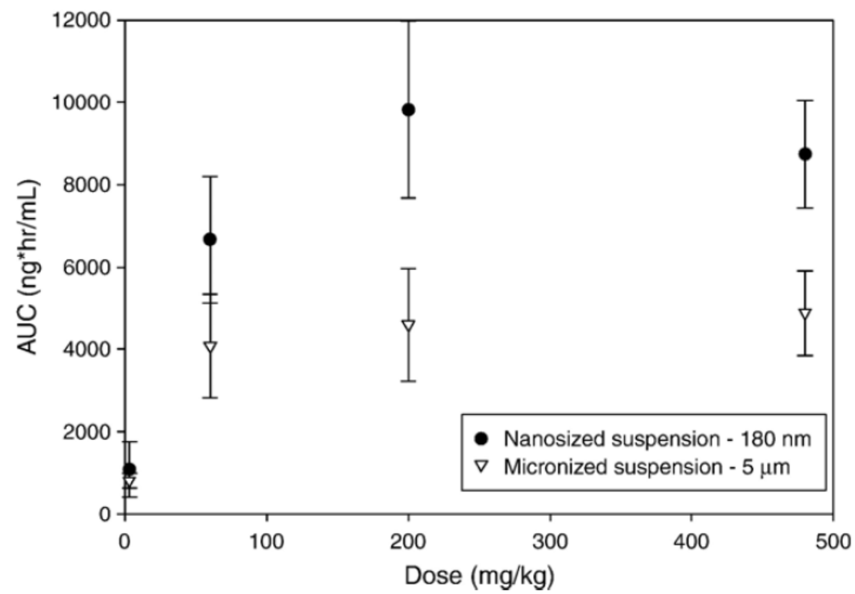


Oberdorster et al. 2005

Enhanced Absorption of Nanoparticles in the Gut

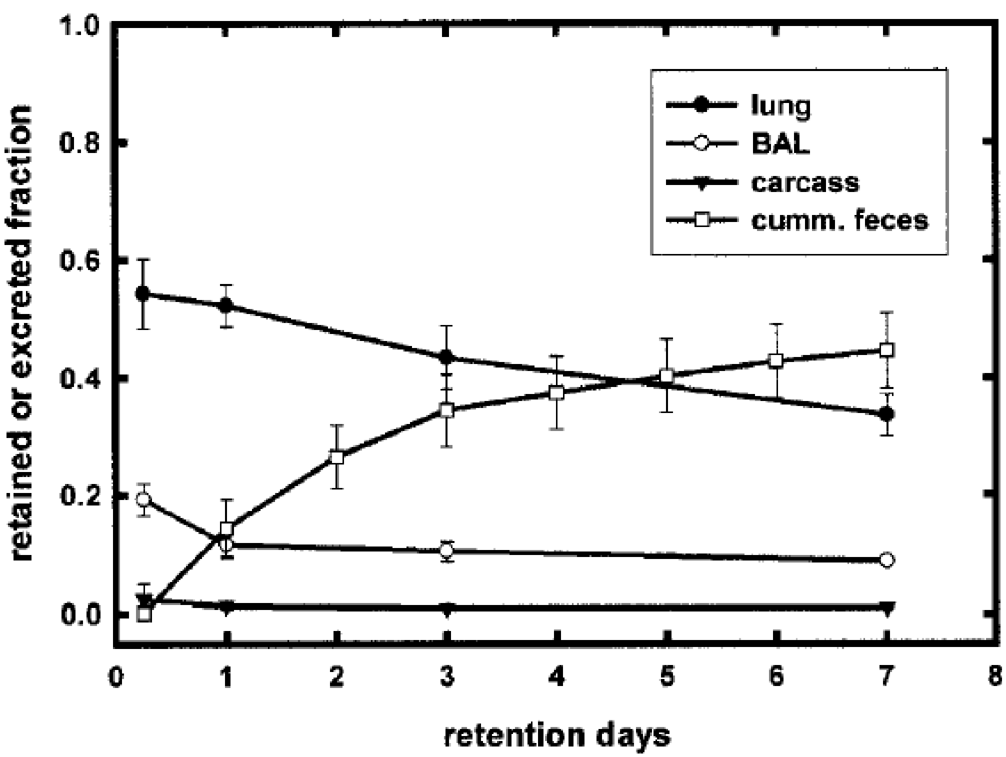
- GI receives ~30-50% of inhaled nanoparticles through clearance via the mucociliary escalator
- Some occupational exposures associated with oral or GI cancers
- Particle size, charge, lipid solubility influences
  - Dissolution rate
  - Bioavailability

Oral Absorption of Nano and Micro Drug Formulations



Kesisoglou et al. 2007 Adv Drug Delivery Rev 59:631 (figure); Jung et al. 2000 Europ J Pharm Biopharm 50:147

Nanoparticles Can Transport to Extrapulmonary Organs

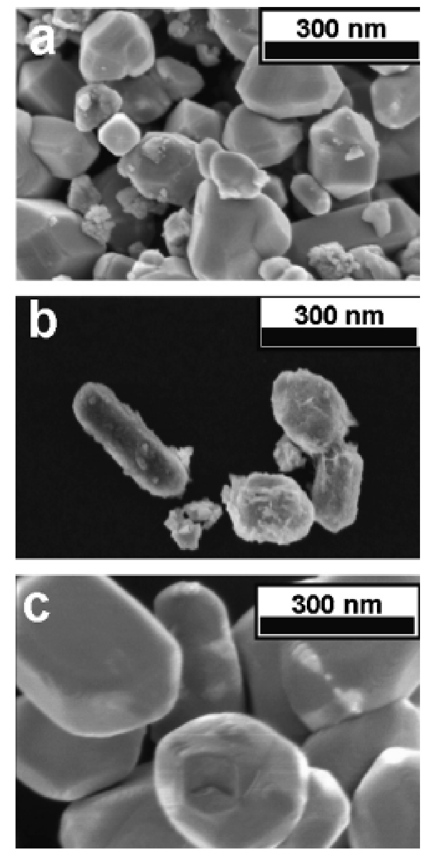


- Retention/excretion of 15 nm Iridium particles
- Peak dose of 0.1-0.5% at 1 week in liver, spleen, kidneys, heart, and brain
- Thereafter declined to 0.01-0.05%
- Although low extra-pulmonary transport is inversely related to particle size

Kreyling et al. 2002 JTEH 65:1513

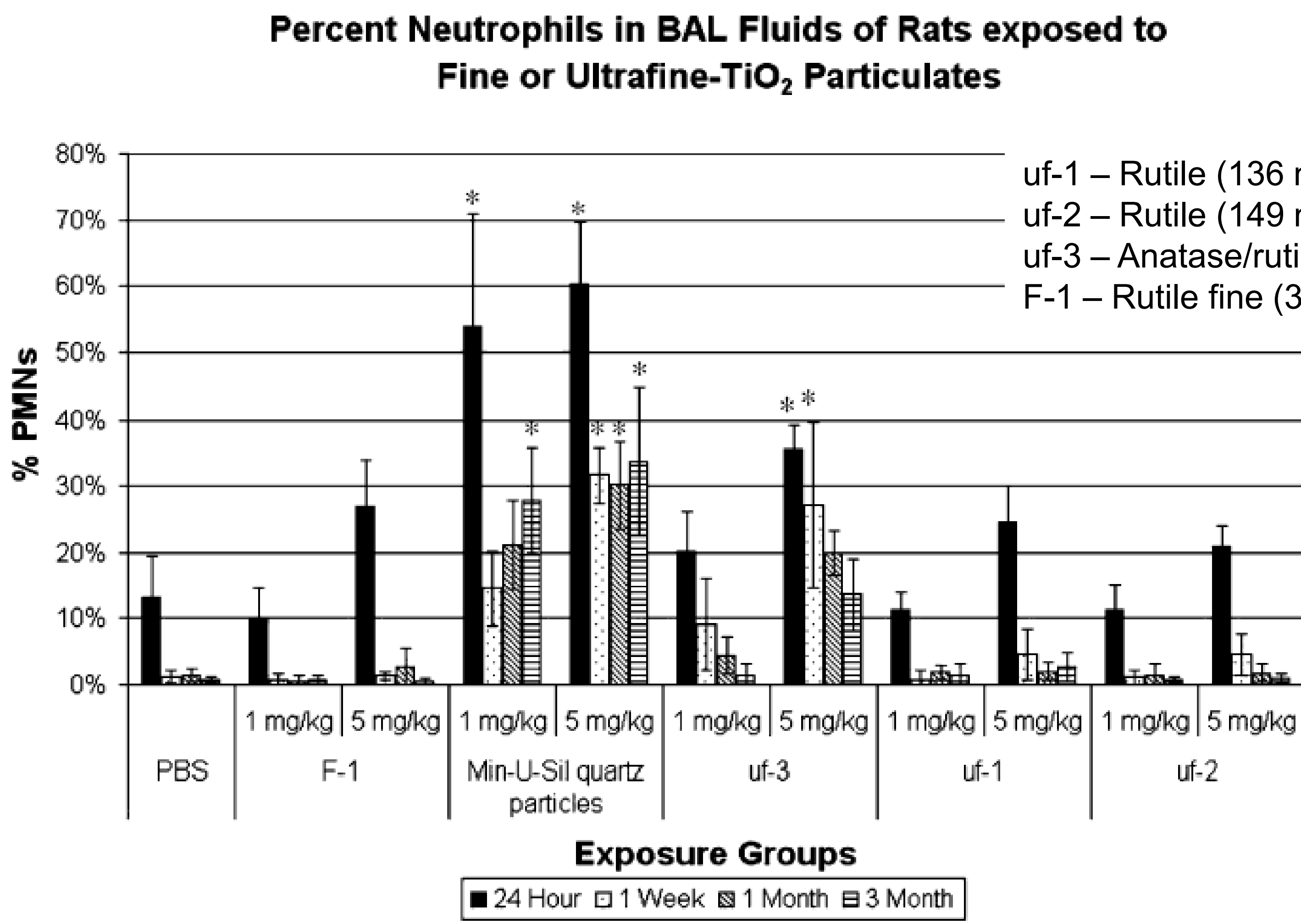
Titanium Dioxide is a Useful Model for Understanding Effects of Nanoparticle Physicochemisty

- Applications
  - Opacifier for paints, coatings, food, and cosmetics
  - UV absorber in sunscreens
- Differential pulmonary toxicity
  - Particle size
  - Crystalline form
  - Agglomeration
  - Surface area
  - Morphology
  - Coatings



Warheit et al. 2007

Toxicity Studies of TiO_2: Effect of Size and Crystalline Form



uf-1 – Rutile (136 nm)
uf-2 – Rutile (149 nm)
uf-3 – Anatase/rutile (25 nm)
F-1 – Rutile fine (382 nm)

Warheit et al. 2007

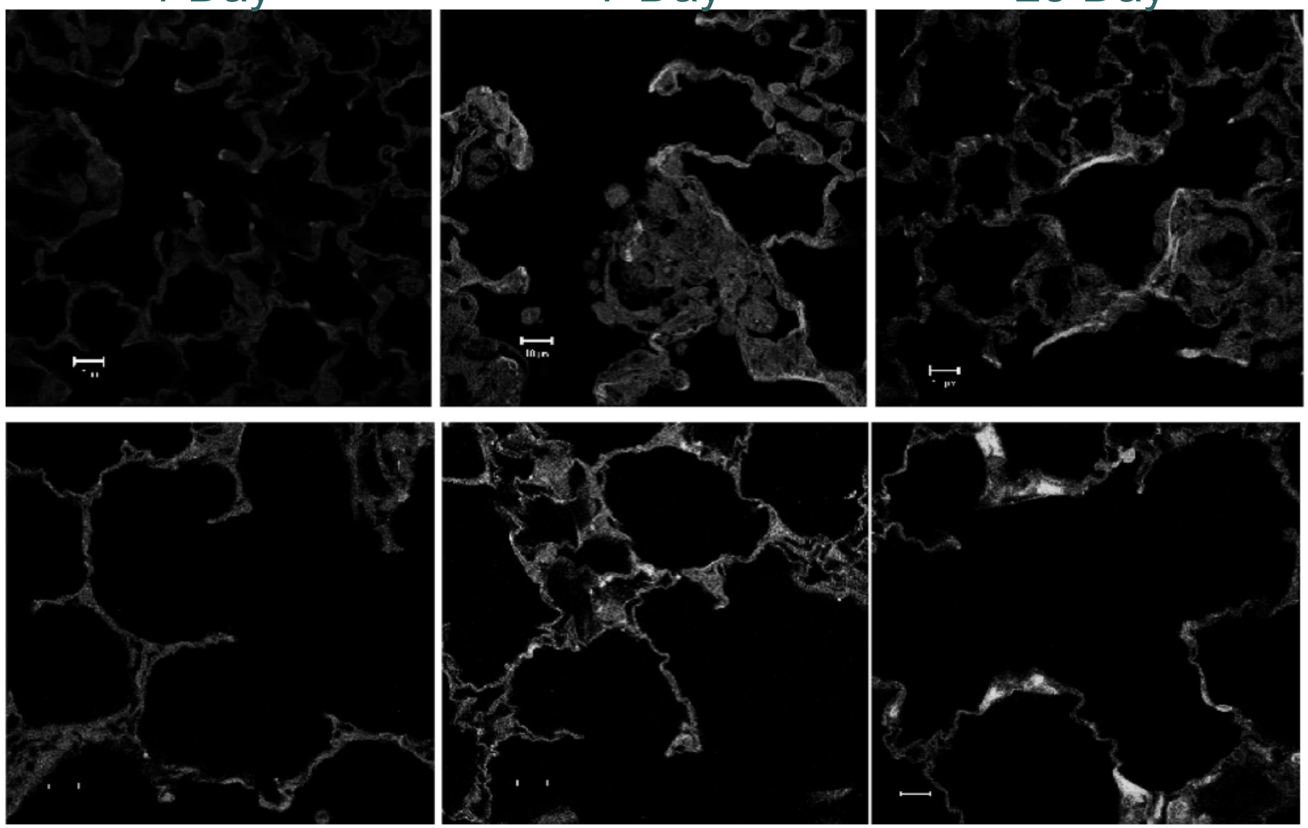
Collagen Response from SWCNTs Can Vary Depending on Extent of Agglomeration

Post-Exposure

1 Day | 7 Day | 28 Day

SWCNTs

Dispersed SWCNTs



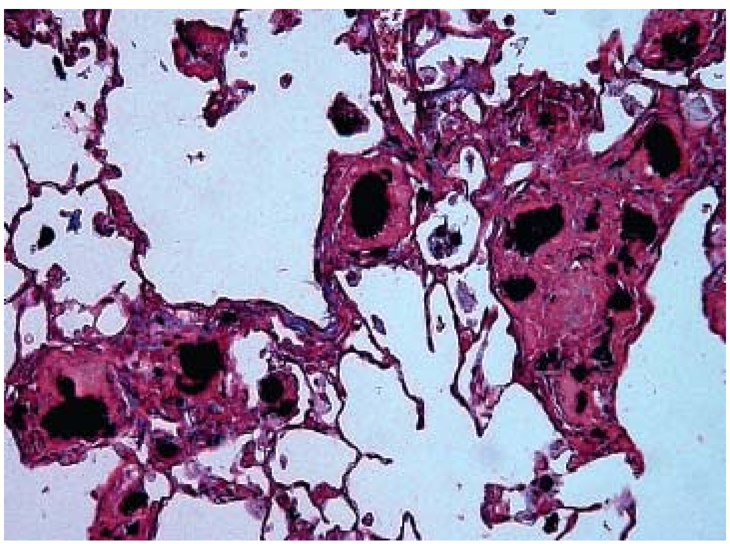
Mercer et al. 2008

MWCNTs Show Greater Responses from Instillation

Instillation of MWCNTs

(Muller et al. 2008)

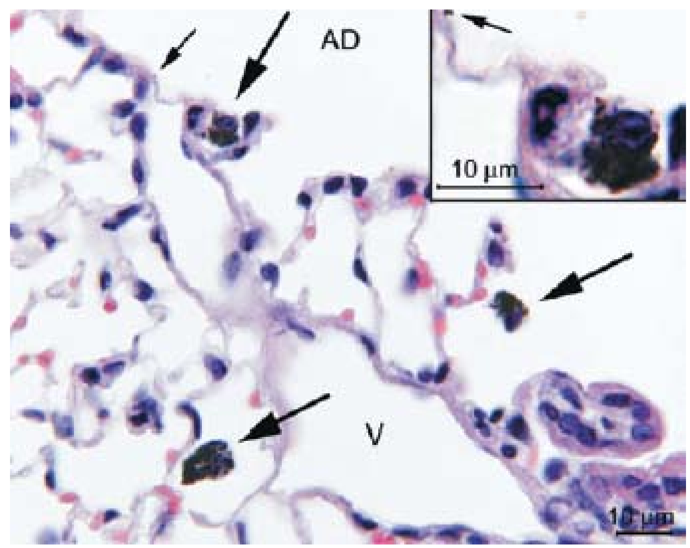
- Single bolus dose of 2 mg per animal



Inhalation of MWCNTs

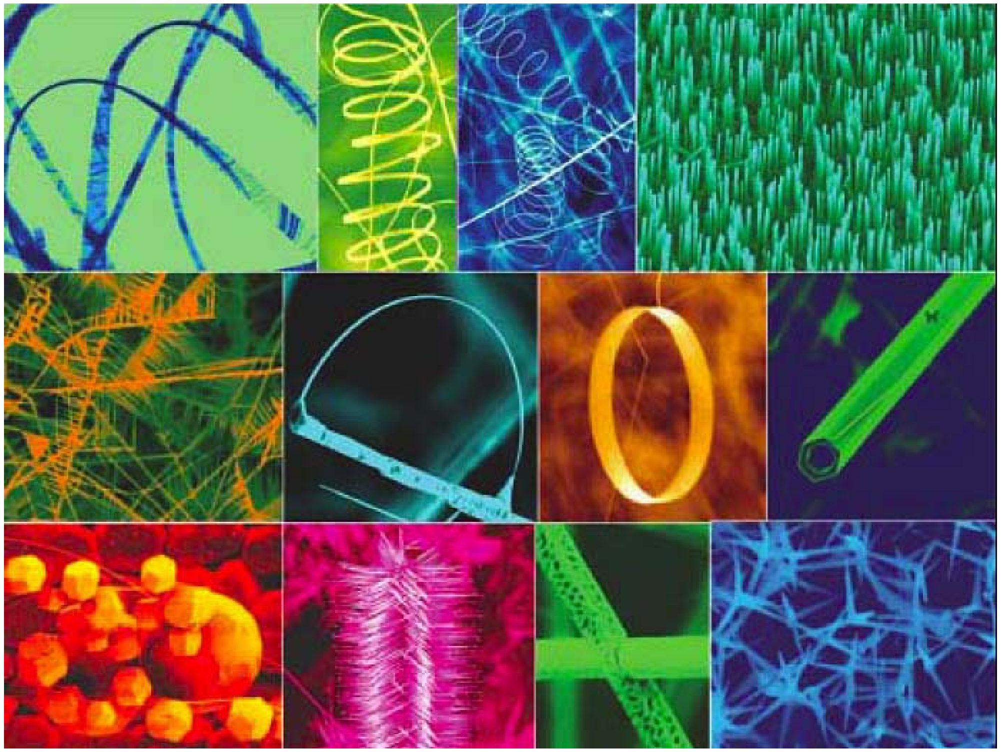
(Mitchell et al. 2007)

- 0.3, 1 or 5 mg/m^3 for 7 or 14 day



Mitchell Toxicol Sci 100 203 2007; Muller Chem Res Tox 2008

Shape



Materials Today June 2004. Zhong Lin Wang, Georgia Institute of Technology

Shapes and Dimensions Vary Greatly for Nanomaterials

- Morphological variations include
  - Solid v. porous
  - Spherical v. tubular
  - Singlet v. agglomerated
- Particle dimensions that inhibit normal clearance mechanisms and increase retention are of greatest concern
- Impaired particle clearance by macrophages (e.g., frustrated phagocytosis, volumetric loading) can play an important role in pathogenesis

Asbestos Fiber Type and Dimension Influences Retention

Fiber Half Times (Days)

Fiber	<5µm	5-20µ	>20µm
Amosite	NR*	NR	418[1]
Crocidolite	44	(234)(142)	817[1, 2]
Canadian Chrysotile	107	29.4	16[3]
U.S. Chrysotile	NR	NR	<1[3]
Chrysotile (Cana-Brava Mine)	>2.4	2.4	1.3[4]
Tremolite	NR	NR	Very Long[3]

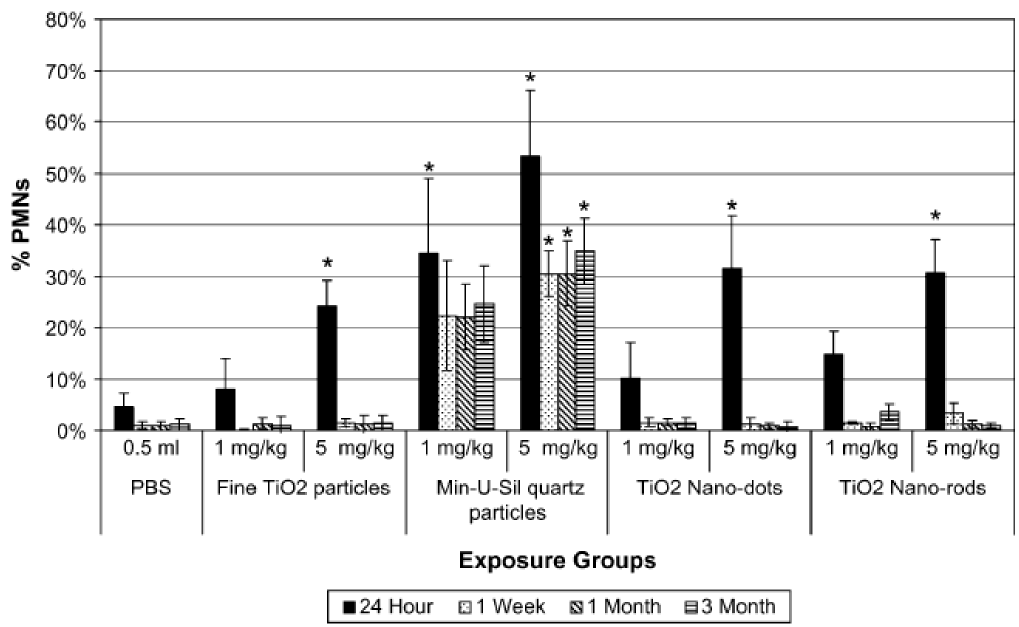
*Clearance of fibers <5µm was faster than Clearance of fibers >20µm.

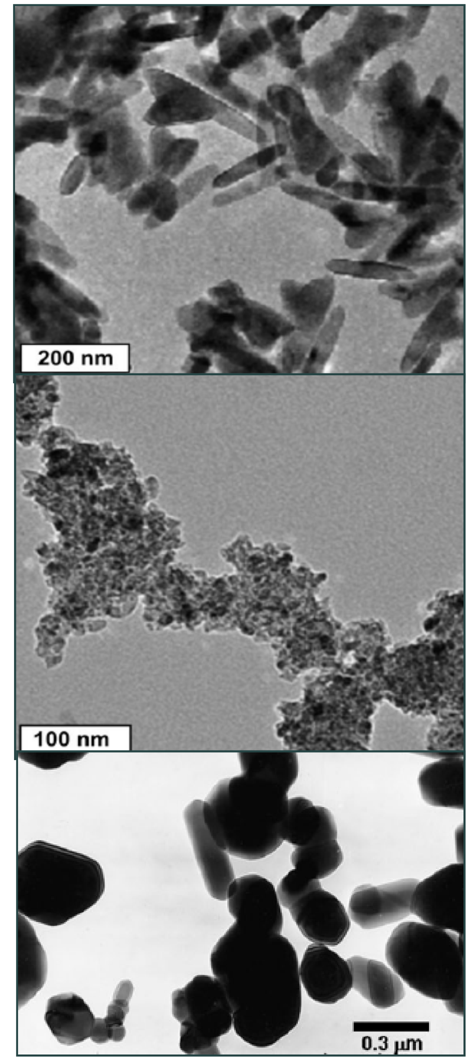
[1] Hesterberg, 1998
[2] Hesterberg, 1996
[3] Bernstein, 2003a, 2003b
[4] Bernstein 1999, 2000

Presented by Dr. David Bernstein at Asbestos Mechanisms of Toxicity Workshop, Chicago, IL 2003

TiO_2 Shape, Size and Surface Area Show Similar Toxicity

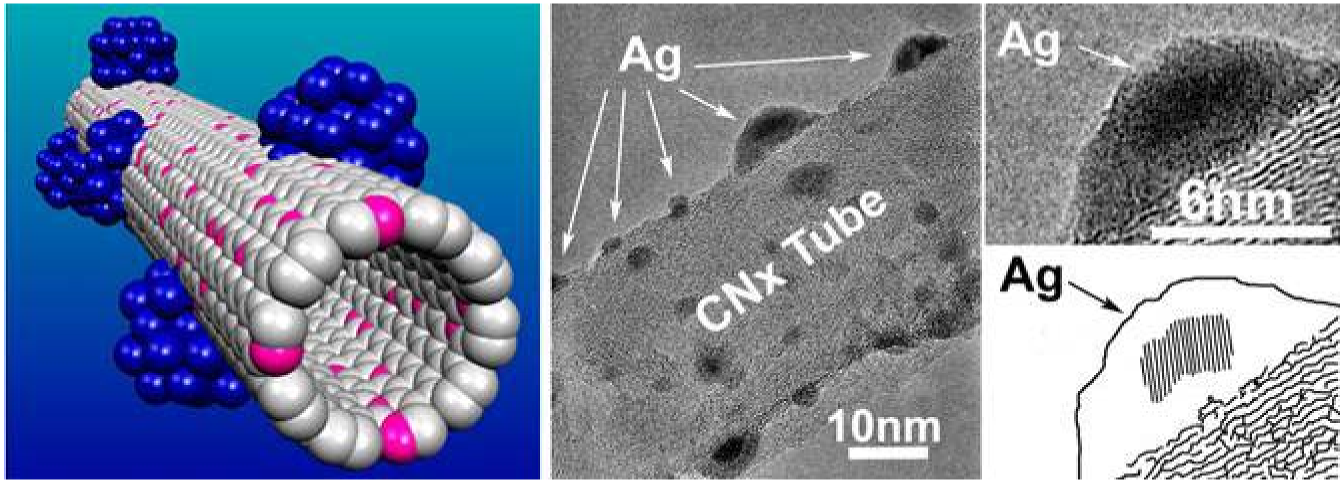
- Nanosized TiO_2 rods and dots show similar effects as fine-sized TiO_2





Warheit Toxicol Sci 91 227 2006

Surface Metals



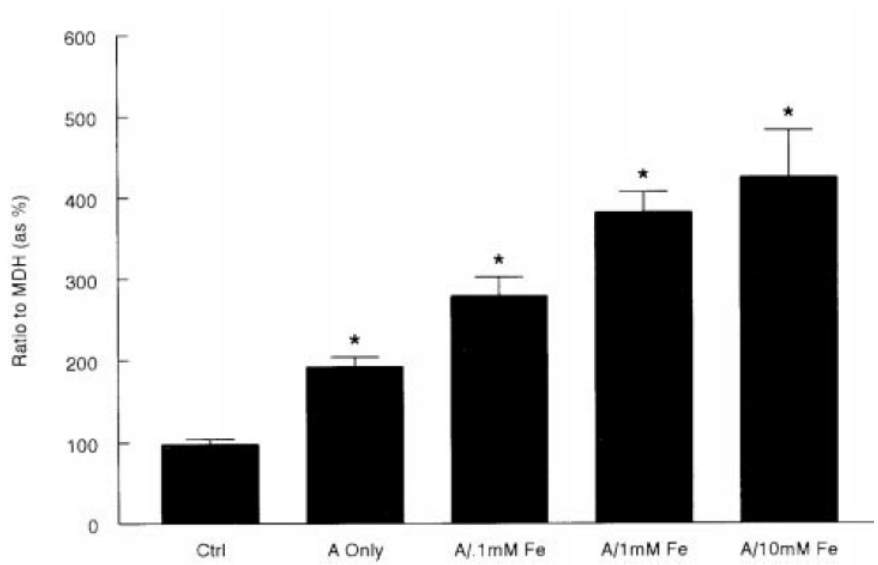
Mauricio Terrones. Recent Advances on N-doped Carbon Nanotubes and their Applications.
http://met.iisc.ernet.in/~nano2006/AbstractSubmissions/Invited.html

Transition Metals

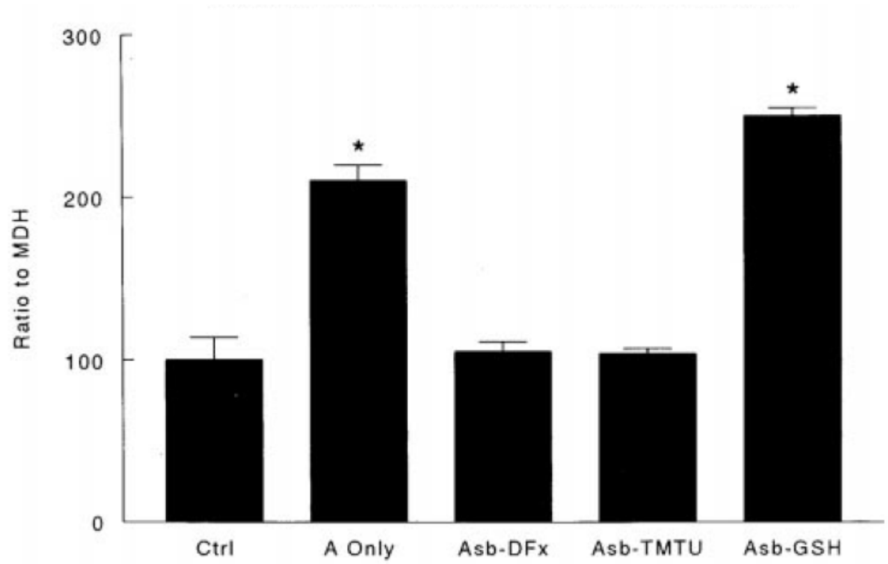
- Catalyze generation of reactive oxygen species, which can lead to
  - Lipid membrane damage
  - Inflammation
  - Oxidative stress
  - Cellular remodeling, necrosis, apoptosis
  - Oncogene upregulation
- Shown to play an important role in the toxicity of asbestos

Iron in Asbestos Increased Profibrotic Markers

Increasing iron content increases procollagen mRNA



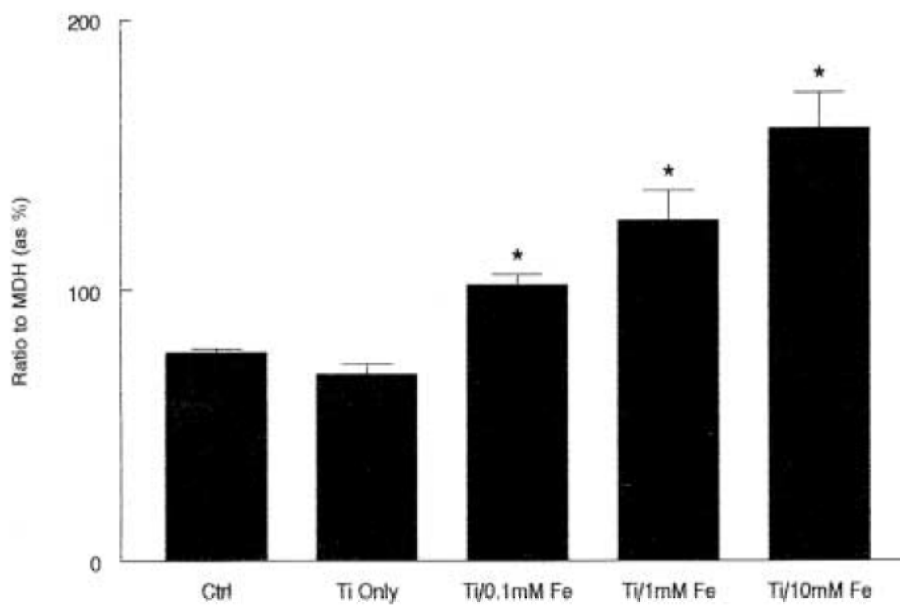
Adding a chelator for iron reduces procollagen mRNA



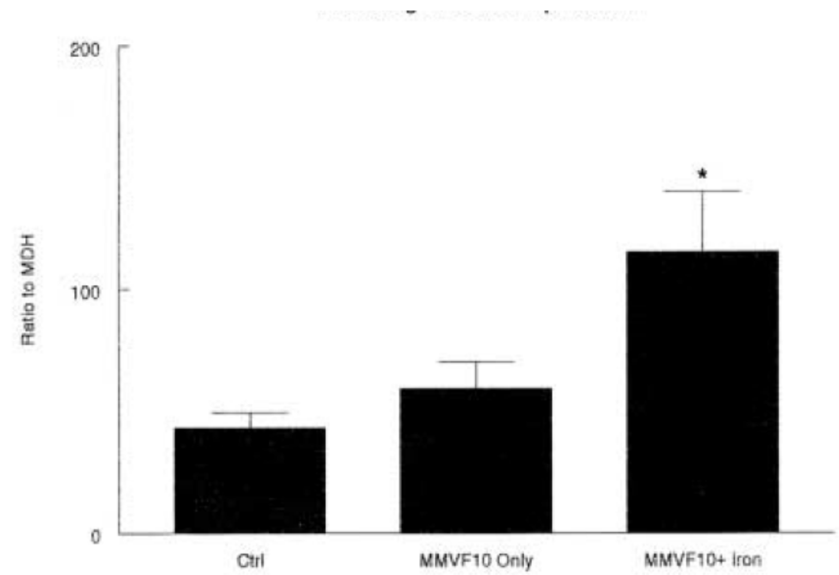
Dai and Churg Am J Respir Cell Mol Biol 24 427 2001

Iron Can Make Nonfibrogenic Particle Fibrogenic

Adding iron to TiO_2 particles increases procollagen mRNA

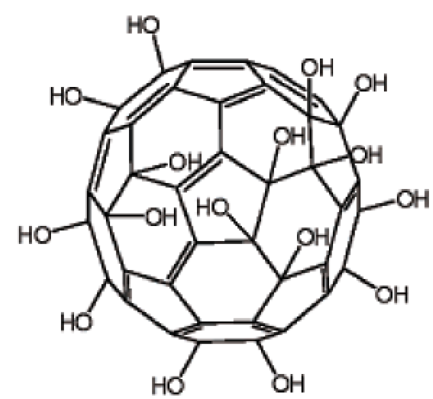


Adding iron to fibers (MMVF) increases procollagen mRNA



Dai et al. Am J Resp Cell Mol Bio 26 685 2002

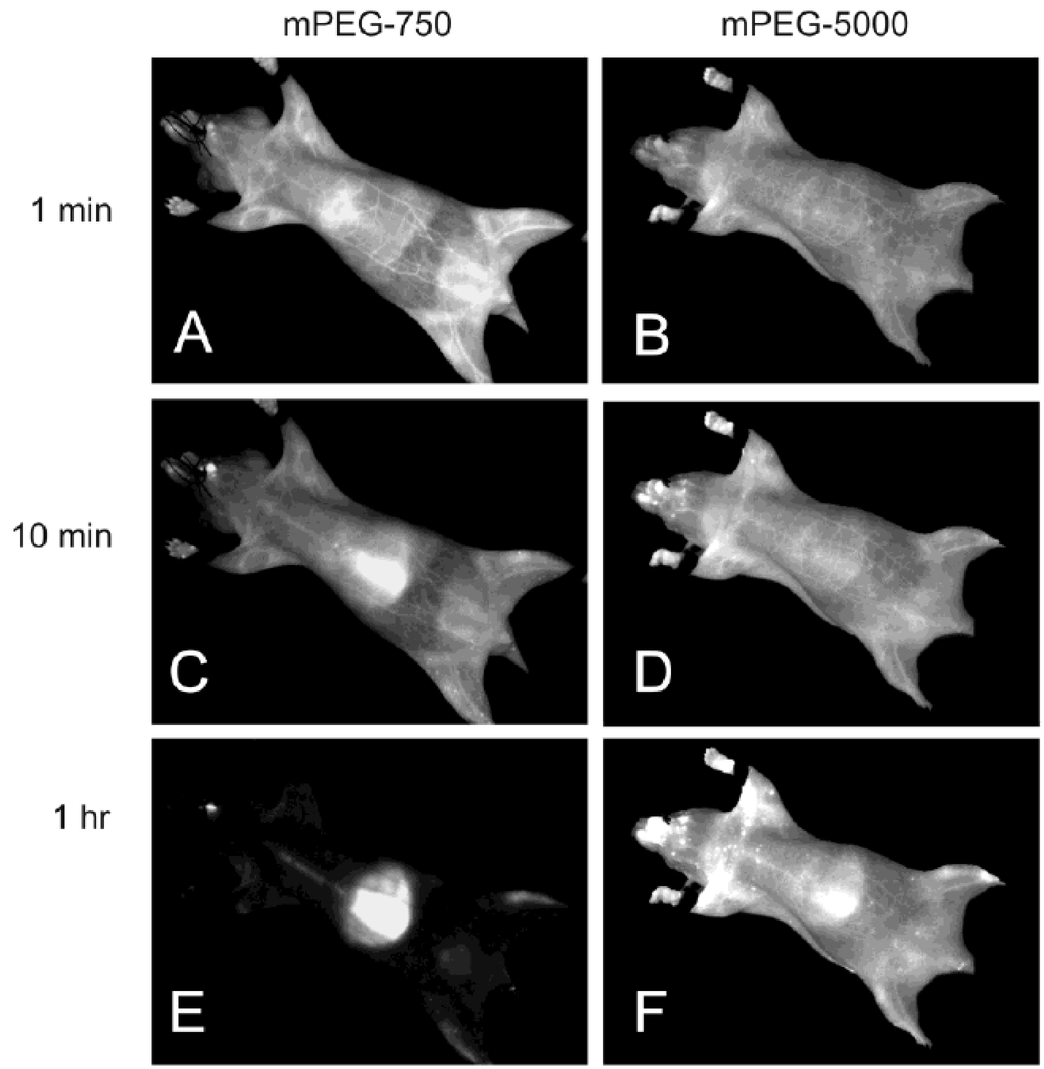
Surface Chemistry and Reactivity



Surface Chemistry Can Effect ADME and Toxicity

- Nanoparticle surface chemistry is a immense area of research for therapeutic applications
- Surface chemistry can effect
  - Absorption, distribution, metabolism, and elimination (ADME)
- Altering this surface chemistry could be the difference between biocompatibility or toxicity
- Surface chemistry can be easily altered through chemical engineering

Fate and Transport of Quantum Dots: Effect of Surface Chemistry



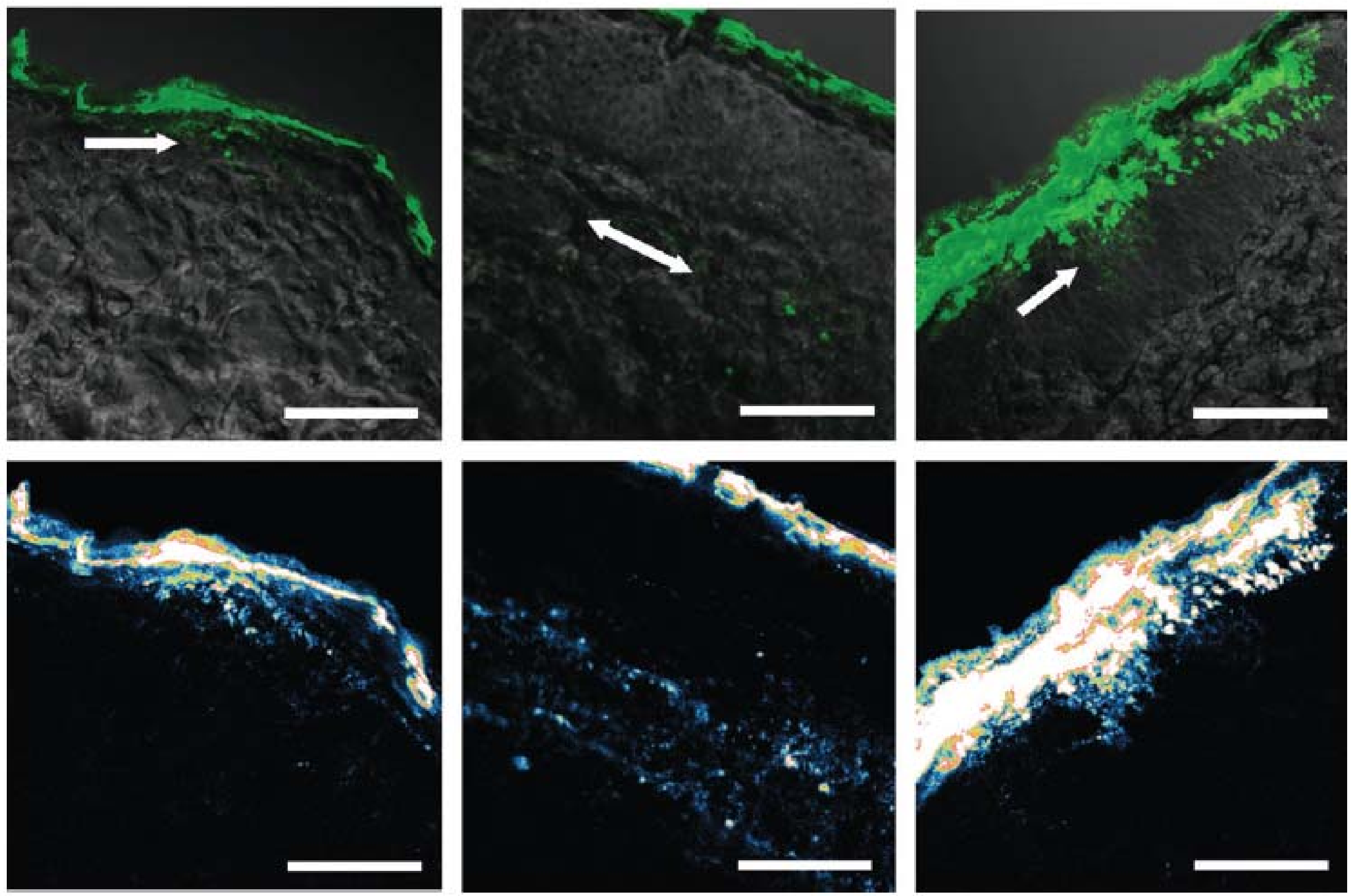
Ballou et al. 2004

Dermal Penetration of Quantum Dots (6nm): Effect of Surface Chemistry

PEG Coated | NH2 Conjugated | COOH Conjugated

QD Localization

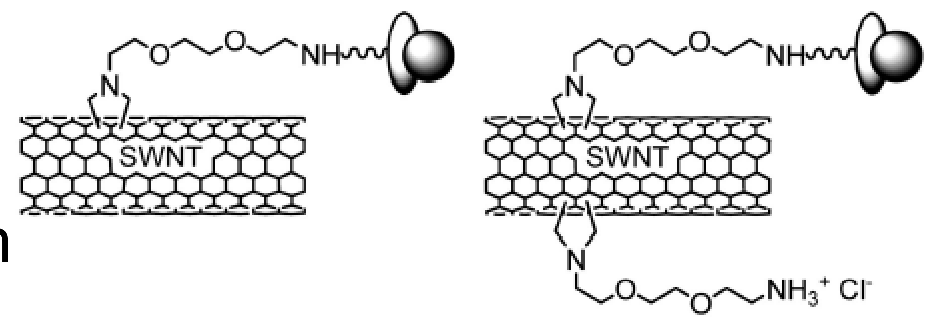
QD Intensity

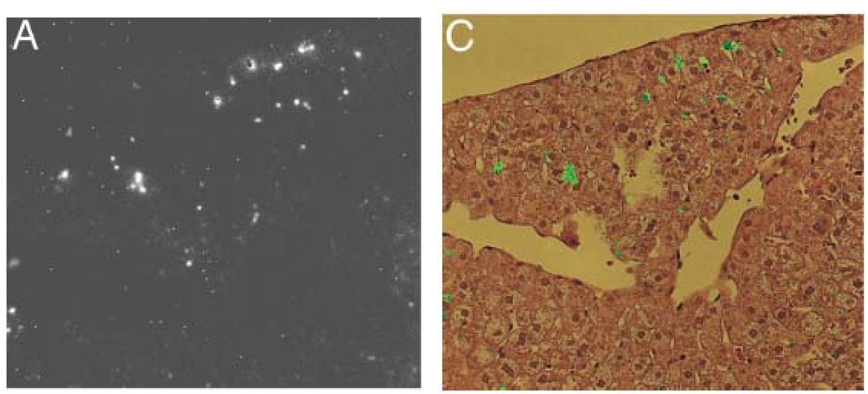


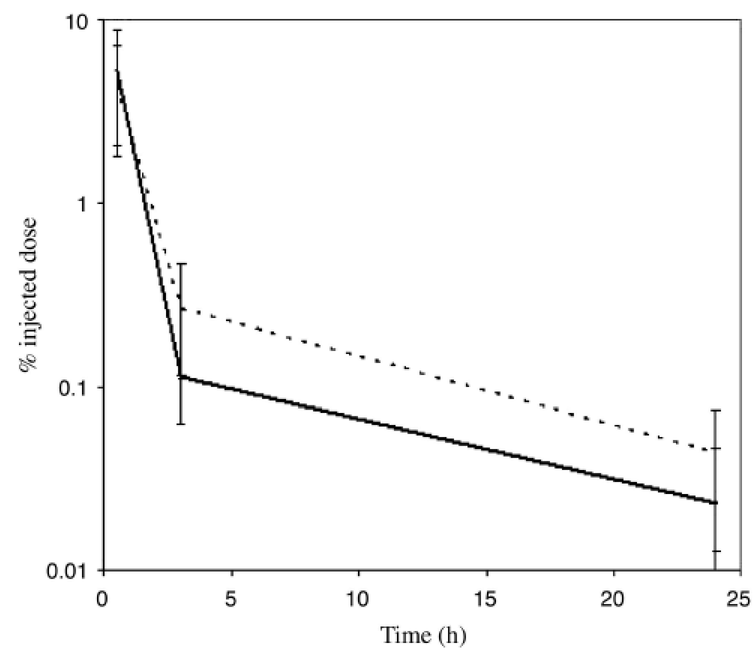
Ryman-Rasmussen et al. 2006

Surface Chemistry Influences Carbon Nanotube Kinetics

- Amine groups cause enhanced excretion
- Non-agglomerated carbon nanotubes show half-life of 1 hr although some accumulation is observed in liver after 24 hr





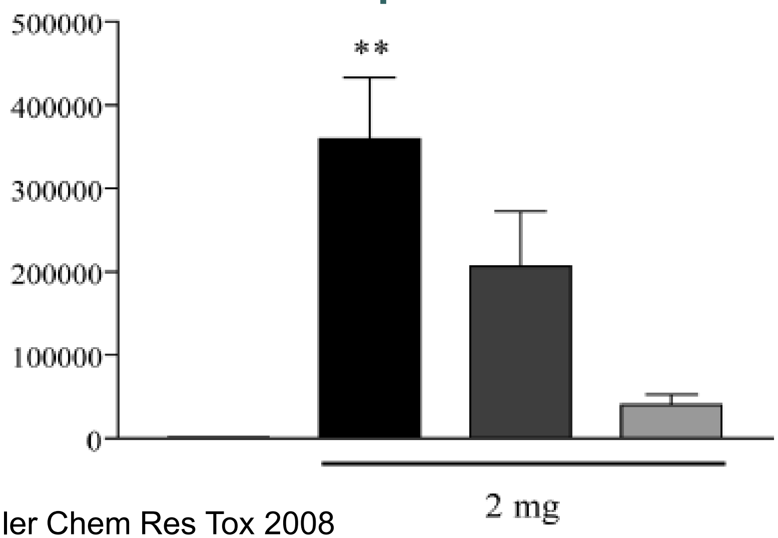


Singh PNAS 103 3357 2006

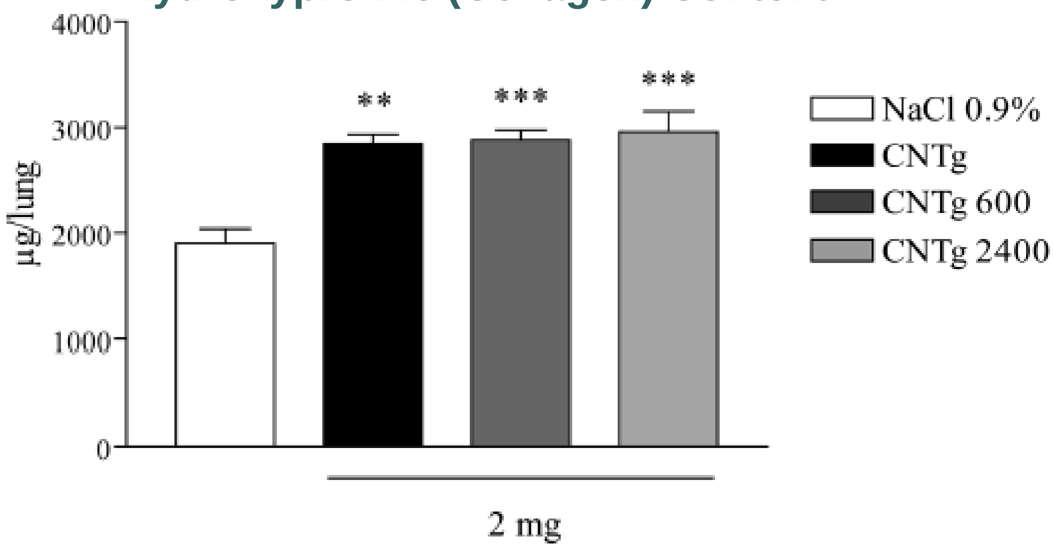
Surface Characteristics of Carbon Nanotubes

- Important surface characteristics driving effects of carbon nanotubes may not be limited to metals, but may include morphology and extent of unpaired or “dangling” bonds

Neutrophil Number

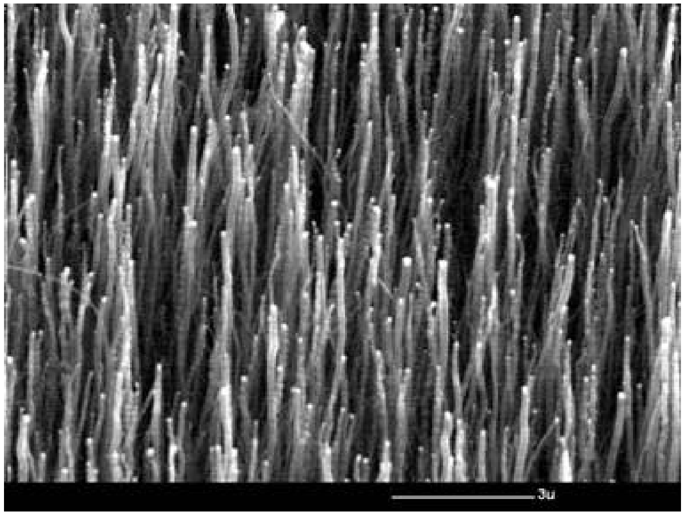


Hydroxyproline (Collagen) Content



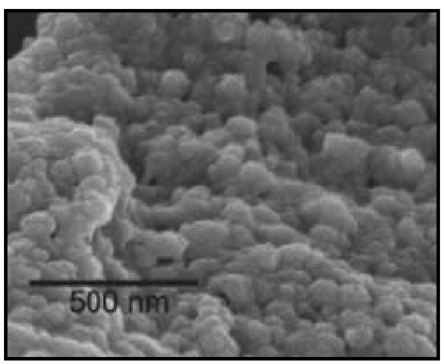
Muller Chem Res Tox 2008

Surface Area

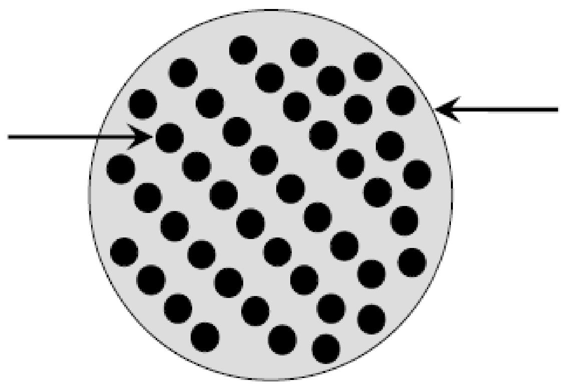


Does Surface Area Tell the Entire Story?

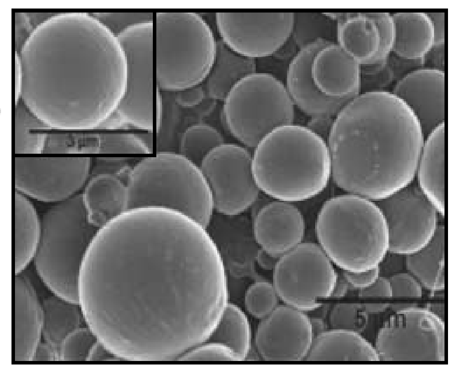
- As particle size decreases, surface area increases, which offers a greater surface for particle-cell interactions…but does this mean it is the only exposure of concern?



Particle Size	~ 200 nm
DNA Loading Efficiency	93-98%



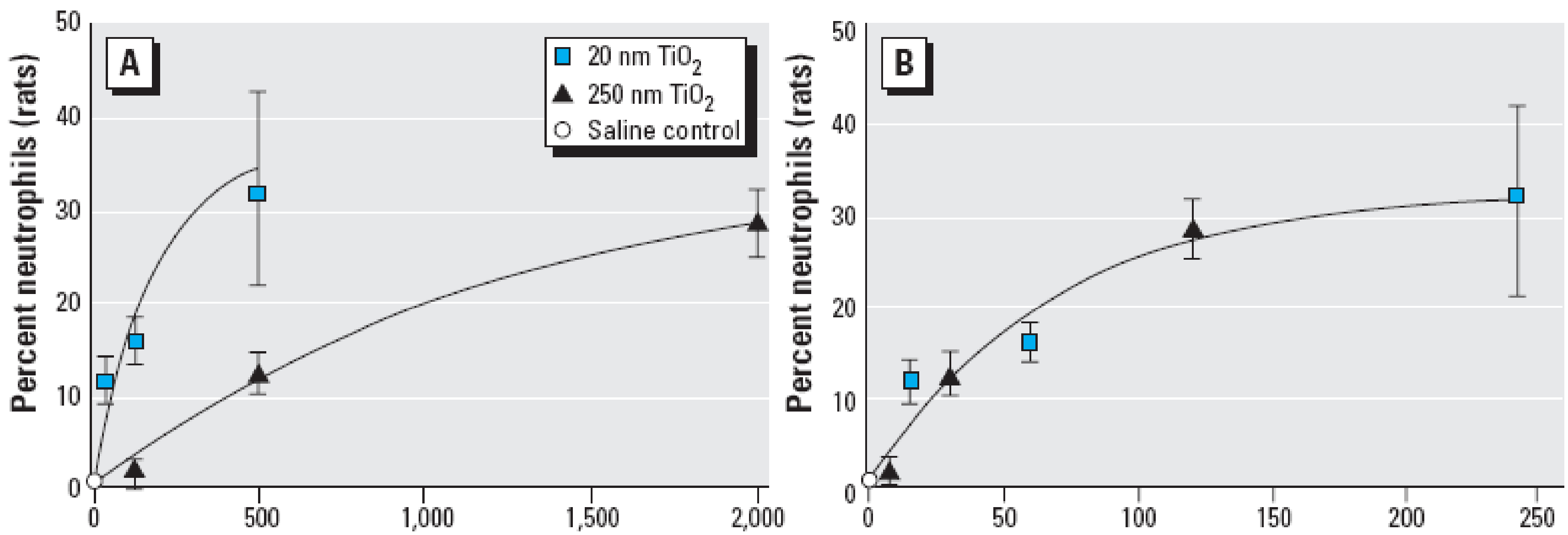
Cross Sectional View of NiMOS



Particle Size	2 -5 µm
DNA Loading Efficiency	~ 46 %

Bhavsar and Amiji Gene Therapy 15:2000 (2008)

Particle Mass/Surface Area and Inflammation

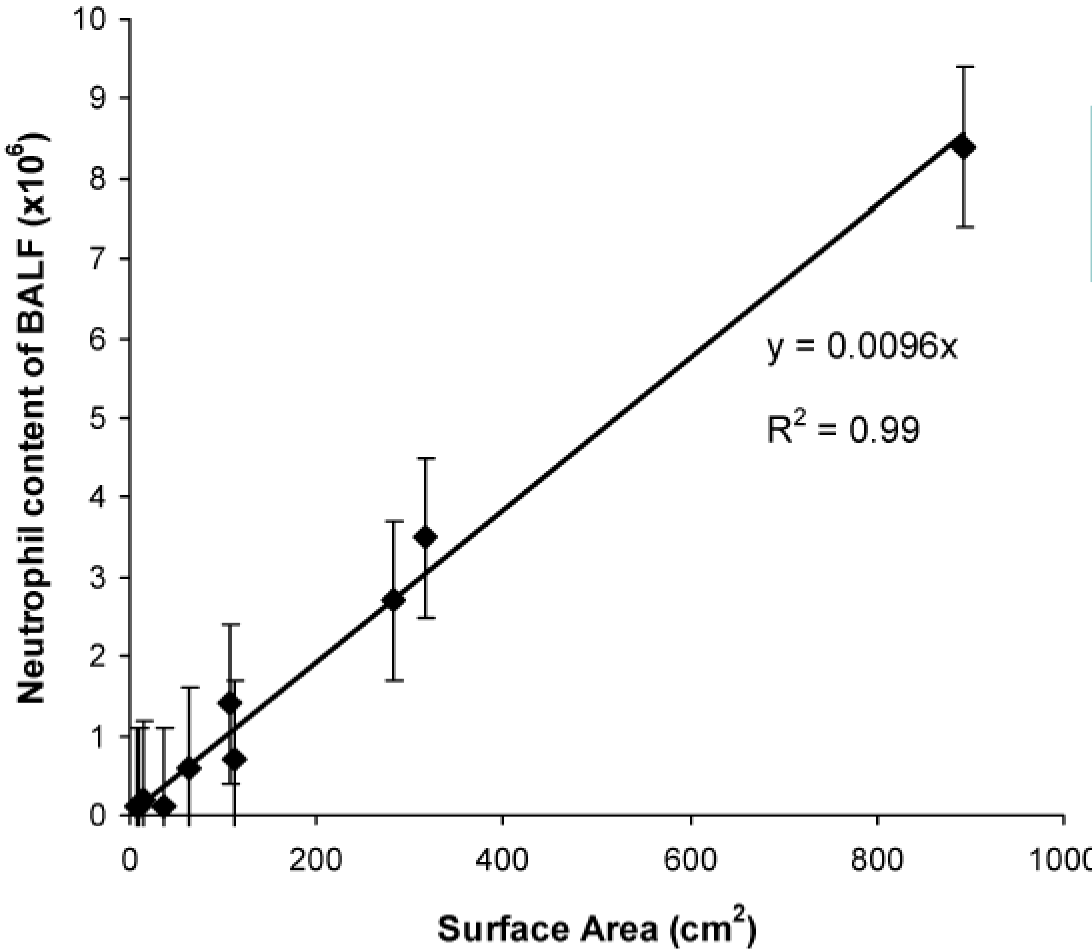


TiO_2 Mass

TiO_2 Surface Area

Oberdorster et al. 2005

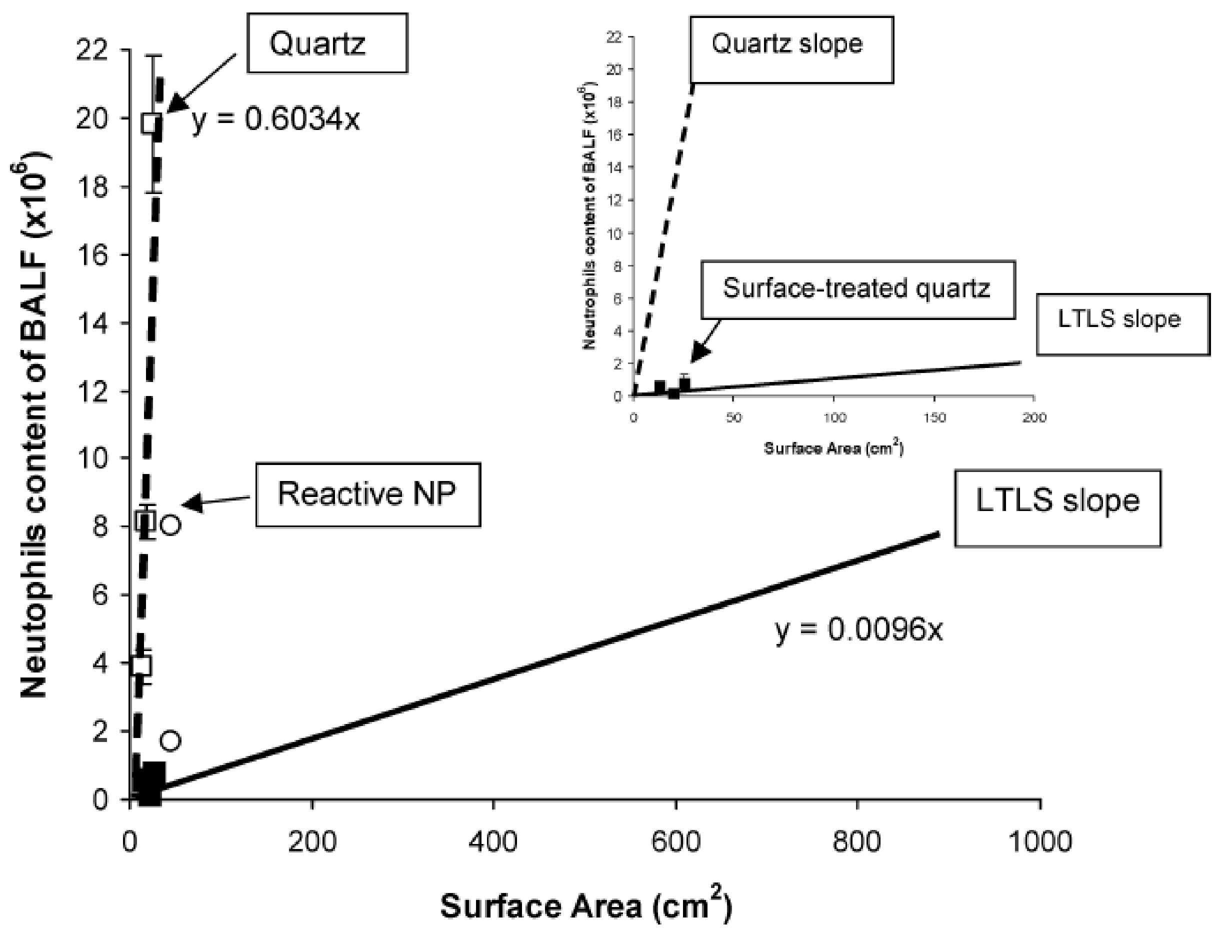
Low Toxicity Particles Follow Similar Dose-Response Pattern



Titanium dioxide
Carbon black
Polystyrene beads

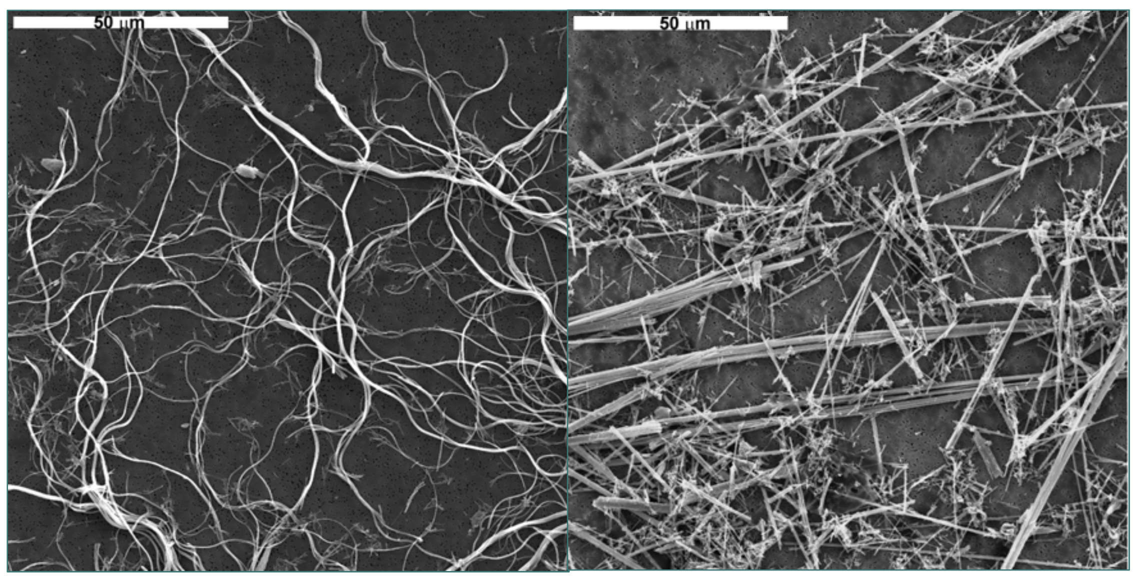
Duffin et al. Inhal Toxicol 19 849 2007

Different Surface Reactivity Changes Dose-Response Curve



Duffin et al. Inhal Toxicol 19 849 2007

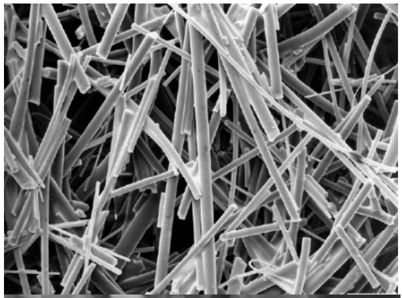
Biopersistence

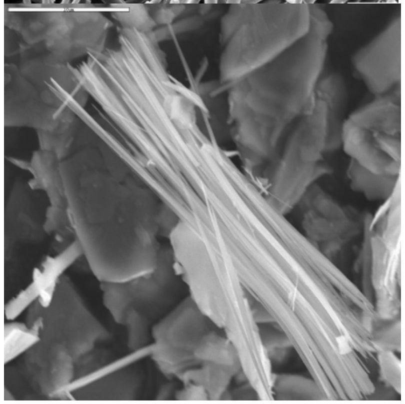


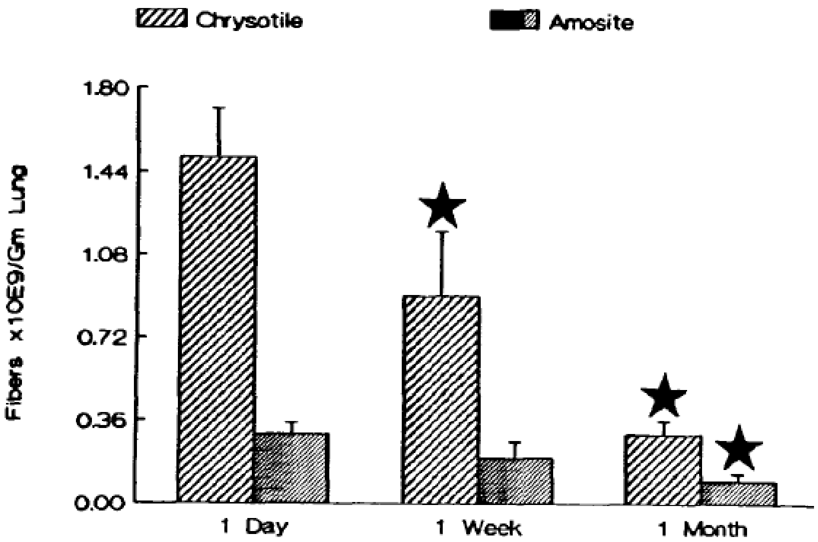
US Geological Survey. http://usgsprobe.cr.usgs.gov/picts2.html

Biopersistence

- Fiber toxicology research has shown that biopersistence (retention + durability) is an important component of toxicity
  - Dimension
  - Durability
  - Dose



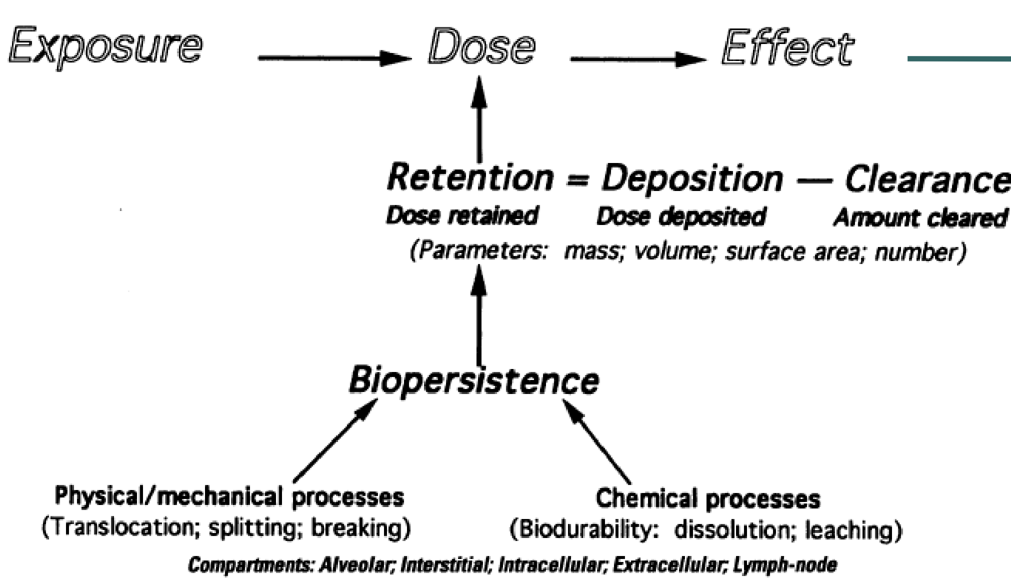




Geiser et al. EHP 111 895 2003; USGS http://usgsprobe.cr.usgs.gov/picts2.html; Churg et al. Am Rev Respir Dis 139 885 1989

Possible Mechanisms of Toxicity

Size
Shape
Metal content
Surface chemistry



Oxidant generation
Oxidative stress
Membrane disruption
Mitochondria perturbation
Inflammation
Altered cell cycle regulation
Altered immunity

Protein, DNA, membrane damage
Necrosis/Apoptosis
Proliferation
Fibrosis
Tumor formation
Altered cell/organ homeostasis

Oberdorster EHP 102 173 1994; Nel Science 311 622 2006

Conclusions

- Nanomaterials have an incredible potential for advances in medicine, energy/fuel conservation, electronics, and environmental remediation
- Past experience has shown that agglomeration, size, shape, and surface chemistry seem to be key determinants, but no one property drives toxicity for all particle types
  - Persistence does not necessarily mean toxicity
  - Engineered nanomaterials with high functionality and rapid excretion are more desirable
- Through systematic toxicity testing and intentional physicochemical modifications, nanomaterials can be designed to maximize their societal benefits and minimize their human health risks

Acknowledgement

- Background literature research, as presented here, has contributed to conceptual development of original research of the health effects of inhaled carbon nanotubes as funded by US EPA Star Grant #83171401 and Student Fellowship for University of California Toxic Substances Research & Teaching Program