RISK*e*Learning

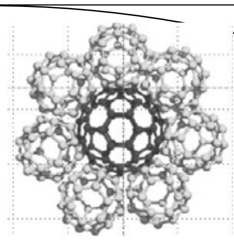
Nanotechnology –
Applications and Implications for Superfund

September 12, 2007
Session 7:
"Nanoparticles: Human Toxicology and Risk Assessment"
Kevin Dreher, US EPA
Agnes Kane and Robert Hurt, Brown University
Stephen Roberts, University of Florida

SBRP/NIEHS
William Suk
Heather Henry
Claudia Thompson
Beth Anderson
Kathy Ahlmark
David Balshaw

EPA
Michael Gill
Jayne Michaud
Warren Layne
Marian Olsen
Charles Maurice
Nora Savage
Barbara Walton
Randall Wentsel
Mitch Lasat
Martha Otto

MDB
Maureen Avakian
Larry Whitson
Larry Reed



Pulmonary Effects of Nanoparticles: Insight Into Challenges Associated with Nanotechnology Health Risk Assessment

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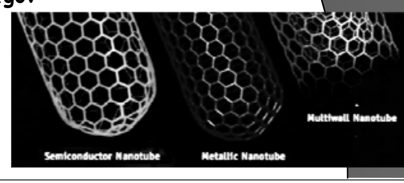
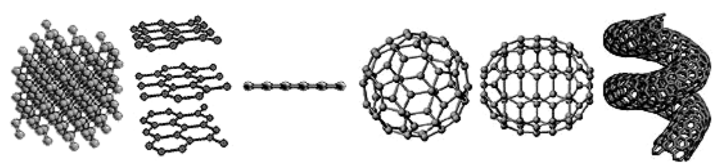


NIEHS Nanotechnology Web Seminar:
Nanoparticle Health Effects
September 12, 2007

Pulmonary Effects of Nanoparticles

Presentation Outline

- Highlight Challenges in Nanoparticle/Nanotechnology Health Risk Assessment
- Pulmonary Toxicology of Nanomaterials:
  - -Dosimetry, Fate, and Effects
  - -Factors Regulating Toxicity
- Extra-Pulmonary Toxicity (Local vs. Systemic)
- Summary



A Gallery of Carbon
from left, Diamond, Graphite, Carbon Chain, C_{60}, C_{70} and Nanotube

1 *Carbon Allotropy* 2

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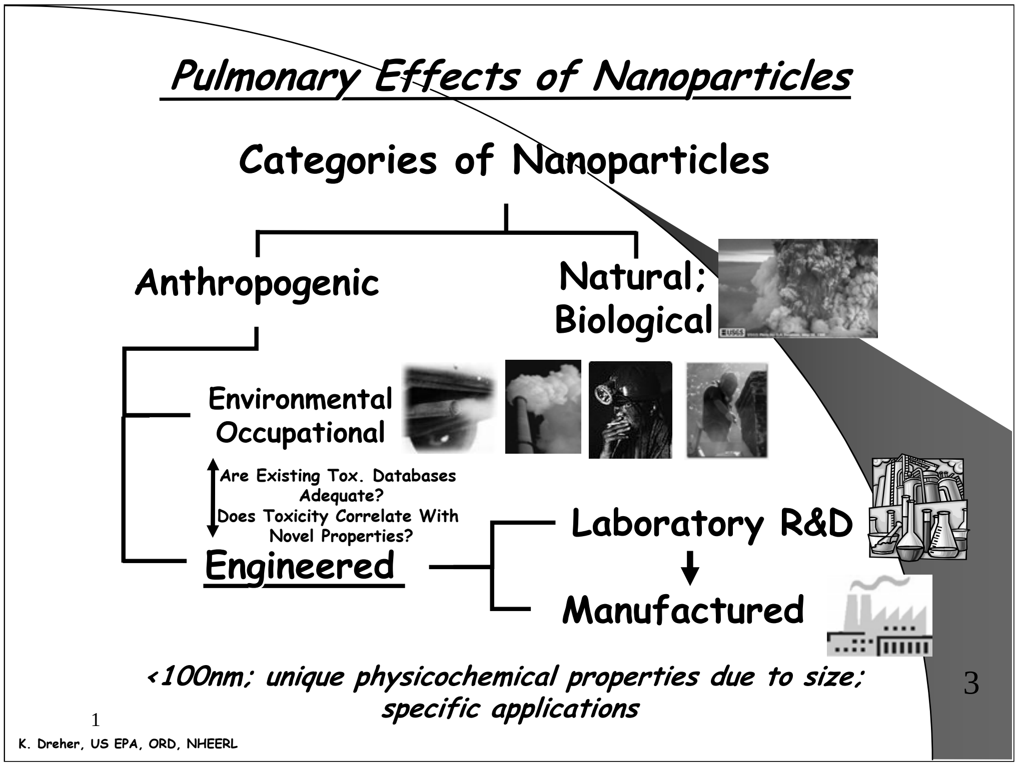
Pulmonary Effects of Nanoparticles
Categories of Nanoparticles
Anthropogenic
Natural;
Biological
Environmental
Occupational
Are Existing Tox. Databases Adequate?
Does Toxicity Correlate With Novel Properties?
Engineered
Laboratory R&D
Manufactured
<100nm; unique physicochemical properties due to size; specific applications
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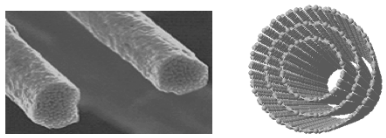
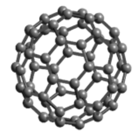
Pulmonary Effects of Nanoparticles

Challenge: Diversity of Nanoparticles

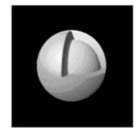
Single and multi walled nanotubes



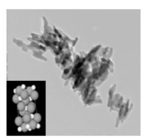
Fullerenes



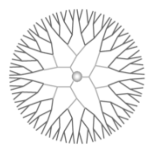
Nanoshells



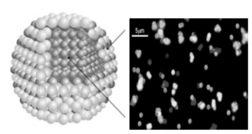
Metal oxides



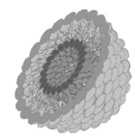
Dendrimers



Quantum dots



Nanosomes



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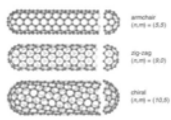
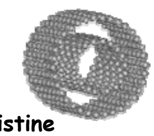
Pulmonary Effects of Nanoparticles

Challenge: Complexity Within Nanoparticle Classes



Carbon Based Nanomaterials



SWCNT & MWCNT
- Laser Ablation → pristine (60%/>90% Purity)* / derivatized
- Gas-Phase Catalytic Process (HiPco) (Fe; Ni; Co; Y; Mo) → * pristine / derivatized
- Chemical Vapor Deposition → * pristine / derivatized
- Electric Arc → * pristine / derivatized

Nanometals / Nanometal Oxides

nZVI
- Toda America → pristine / derivatized
- Zhang - Lehigh Univ. → pristine / derivatized

$nTiO_2$*
- rutile → pristine / derivatized
- anatase → pristine / derivatized**
- amorphous → pristine / derivatized

*, 2 production methods
**, doped (V; Nb)

$nCeO_2$ – five different production methods

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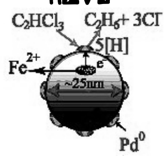
Pulmonary Effects of Nanoparticles

Challenge: Indirect Health Effects of Nanotechnology
(Production and Use of Nanomaterials)

nZVI — $nTiO_2$ — $nCeO_2$



Remediation **Air Pollution Control/Energy**

⬇

Interactions with Environmental Media (Air, Water, Soil)?

⬇

Transformation(s), and Potential Health Effects?

Diesel Exhaust "non-nano" Cerium Additive:

- ↑ >50% in each: benzene; 1,3-butadiene; acetaldehyde (Air Toxics*)
- ↓ 80% PAHs (Air Toxic)
- ↓ 8-20% NOx (NAAQ*)
- ↑ 50-100% CO (NAAQ)
- ↑ Ambient Air Levels of Ce (Predicted)

*Changes in regulated air pollutants

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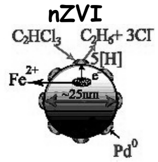
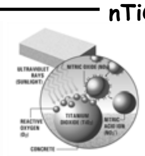
Pulmonary Effects of Nanoparticles

Challenge: Indirect Health Effects of Nanotechnology

(Production and Use of Nanomaterials)

nZVI **nTiO$_2$** **nCeO$_2$**



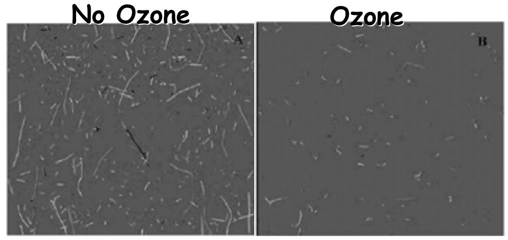


Remediation **Air Pollution Control/Energy**

Interactions with Environmental Media (Air, Water, Soil)?

Transformation(s), and Potential Health Effects?

No Ozone **Ozone**



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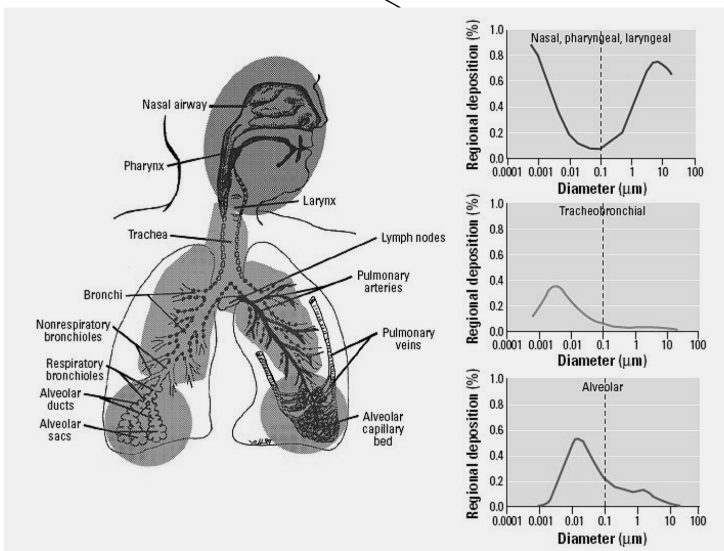
Chen et al., *J. Phys. Chem.*, 110, 2006

Pulmonary Effects of Nanoparticles

Dosimetry: Differential Deposition of Nanoparticles



Oberdorster[3], *EHP*, 2005; Oberdorster et al., *Inhal. Tox.*, 2004

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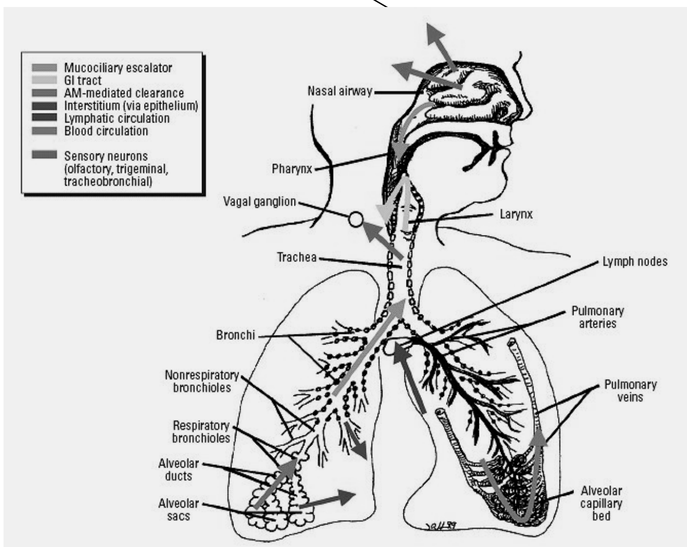
Pulmonary Effects of Nanoparticles

Differential Clearance/Fate (Biokinetics) of Nanoparticles



Oberdorster et al., *EHP*, 113, 2005; Oberdorster et al., *Inhal. Tox.*, 16, 2004

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Pulmonary Effects of Nanoparticles

Challenge: Translocation and Fate in Biological Systems
(Local & Systemic Toxicities)

Rats

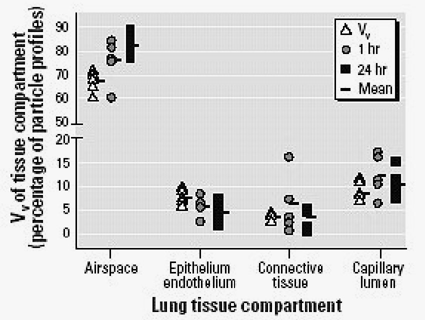

Figure 2. Relative distribution of particles localized in the different lung compartments at 1 hr and 24 hr after inhalation. Volume densities (V_v) for lung tissue compartments from Burri et al. (1973), Pinkerton et al. (1992), and Tschanz et al. (1995, 2003).

Geiser et al., *EHP*, 113, 2005
-nTiO_2, 22nm CMD (4nm primary)
-Inhalation, 110 µg/m^3, 1 h

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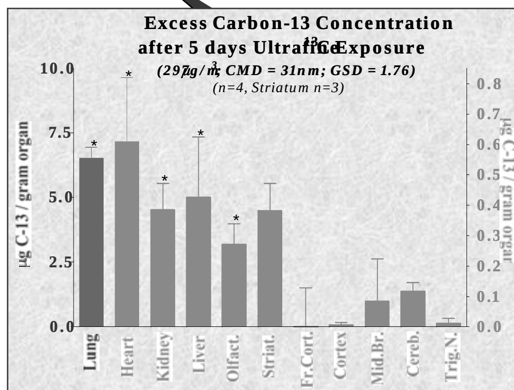
Rats



Oberdorster et al., *Inhal. Tox.*, 2004
Oberdorster et al., *JTEH*, 2002

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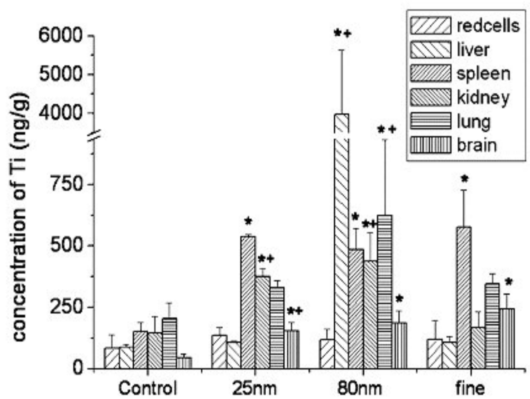
Pulmonary Effects of Nanoparticles

Challenge: Translocation and Fate in Biological Systems

Fate of nTiO2 Following Oral Exposure

- **Wang et al., *Tox. Lett.*, 168:176-185, 2007**
  - **-CD-1(ICR), 19gr, male and female mice**
  - **-oral gavage, 5 gr/kg BW**
  - **-25 nm, 80 nm, 155 nm (fine) TiO_2**
  - **-2 weeks post-exposure**



GI Uptake Distribution Only in Female Mice (Host Susceptibility Factors)

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Pulmonary Effects of Nanoparticles

Challenge: Health Effects/Toxicity of Nanoparticles

(Toxicity and Mechanism(s) of Injury Unique to "Nanoness"; Local vs. Systemic Toxicities; Adequacy of Existing Toxicological Databases)

SWCNT - Toxicity

- **Warheit et al., *Tox. Sci.*, 77, 2004**
  - -Crl:CD(SD) IGS BR, male rats
  - -Crude SWCNT (laser ablation)
  - -Comparative: quartz; graphite; carbonyl iron
  - -IT-instillation, 5 mg/kg BW
  - -3 mon post-exposure
- **Lam et al., *Tox. Sci.*, 77, 2004**
  - -B6C3F, male mice
  - -Crude & Pure SWCNT (HiPCo; Arc Produced)
  - -SWCNT (Arch Generated)
  - -IT-instillation, 0.1, 0.5 mg/kg BW
  - -3 mon post-exposure
- **Shvedova et al., *Am. J. Physiol. Lung Cell Mol. Physiol.*, 289, 2005**
  - -C57BL/6, female mice
  - -Pure SWCNT (HiPCo)
  - -Comparative: silica; nano carbon black
  - -PA; 0, 10, 20, 40 µg/mouse
  - -Up to 60 days post-exposure

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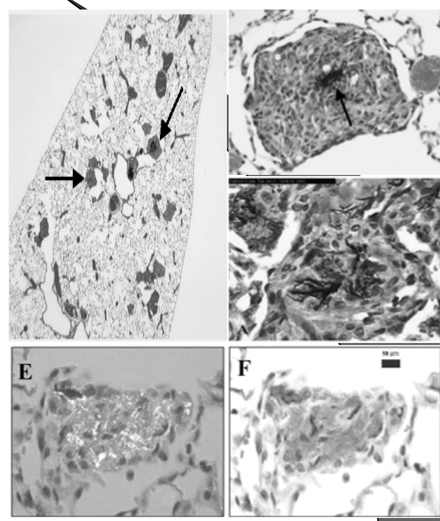
Pulmonary Effects of Nanoparticles

Challenge: Health Effects/Toxicity of Nanoparticles

SWCNT – Results

- Pro-inflammatory and fibrogenic response; increase in cytokines and granuloma formation
- Alteration in pulmonary function
- Decreased pulmonary bacterial clearance
- Comparative toxicological assessment using equivalent mass exposure:
  SWCNT = Quartz >> nano Carbon Black > Graphite
  - -MSDS sheet reference graphite for health hazard specifications
  - -Toxicity unique to "nanoness" (?)
- Pristine (raw) more toxic than purified
  - -Hazard identification – role of metals and oxidative stress (surface reactivity)



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Pulmonary Effects of Nanoparticles

Challenge: Health Effects/Toxicity of Nanoparticles

SWCNT Toxicity Dependent on Production Method

TABLE 2
Incidence of Pulmonary Lesions in Mice 90 Days Aft[er]
Intratracheal Instillation with Nanotubes*

Dust dose (mg)	Type of lung lesion	Carbon black	Quartz	(HiPco) RNT	PNT	(Arc) CNT
0.1	Inflammation	0	1	3	2	0
0.1	Granulomas	0	0	5	2	0
0.5	Inflammation	0	4	3	5	0
0.5	Granulomas	0	0	5	5	5**

Lam et al. *TOX SCI*, 2004

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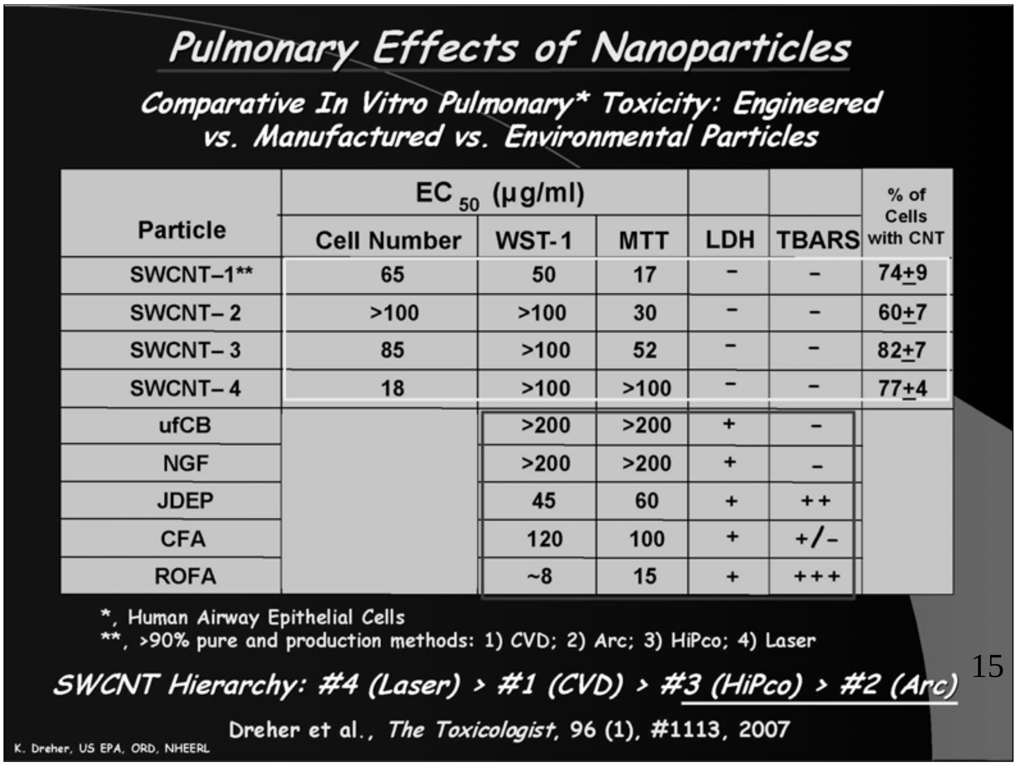

Pulmonary Effects of Nanoparticles

Comparative In Vitro Pulmonary Toxicity: Engineered vs. Manufactured vs. Environmental Particles*

Particle	EC $_{50}$ (μg/ml) Cell Number	WST-1	MTT	LDH	TBARS	% of Cells with CNT
SWCNT–1**	65	50	17	–	–	74±9
SWCNT– 2	>100	>100	30	–	–	60±7
SWCNT– 3	85	>100	52	–	–	82±7
SWCNT– 4	18	>100	>100	–	–	77±4
ufCB		>200	>200	+	–	
NGF		>200	>200	+	–	
JDEP		45	60	+	++	
CFA		120	100	+	+/–	
ROFA		~8	15	+	+++	

Comparative In Vitro Pulmonary* Toxicity: Engineered vs. Manufactured vs. Environmental Particles
SWCNT Hierarchy: #4 (Laser) > #1 (CVD) > #3 (HiPco) > #2 (Arc)
Dreher et al., *The Toxicologist*, 96 (1), #1113, 2007

Pulmonary Effects of Nanoparticles

Challenge: Health Effects/Toxicity of Nanoparticles

MWCNT - Toxicity

- Muller et al., *Toxicol. Appl. Pharmacol.* 207, 2005
  - -Crl:CD(SD), female rats
  - -Purified MWCNT (unground vs. ground)
  - -Unground MWCNT: 5.9μm; 5nm ID; 10nm OD
  - -Ground MWCNT: 0.7μm; 5nm ID; 11nm OD
  - -Comparative: nano-carbon black; asbestos (chrysotile A)
  - -IT-instillation, 0.5, 2, 5 mg/rat
  - -3, 15, 60 days post-exposure
- Carrero-Sanchez et al., *Nano Letters,* 6, 2006
  - -CD1 male mice
  - -Purified MWCNT
  - -CNx-MWCNT
  - -oral, nasal, IT, IP, 1, 2.5, 5 mg/kg BW
  - -Up to 30 days post-exposure

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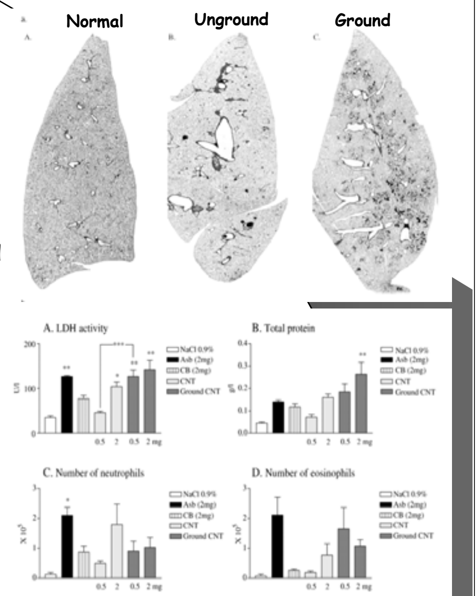
Pulmonary Effects of Nanoparticles

Challenge: Health Effects/Toxicity of Nanoparticles

MWCNT –Results

- Pro-inflammatory and fibrogenic with granuloma formation by ground, unground, and surface unmodified MWCNT
  - -more granulomas/inflammation with ground MWCNT
- Greater clearance of ground vs. unground
- MWCNT more toxic than CNx-MWCNT
  - -Hazard identification – surface reactivity
  - -Toxicity unique to "nanoness" (?)
- Comparative Toxicity:
  - -Asbestos $\geq$ Ground > Unground > nanoCB



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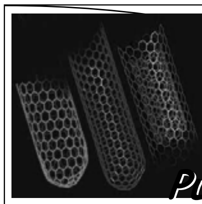
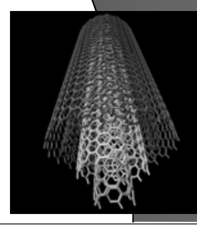


Pulmonary Effects of Nanoparticles

Challenge: CNT Hazard Identification (Multi-Factorial)

Preliminary studies indicate pulmonary toxicity of SWCNTs/MWCNTs is regulated by production method, length, purity (catalysts; substrates), and surface modifications

Do SWCNTs/MWCNTs conform to a fiber paradigm?
-aspect ratio (diameter, length)
-surface reactivity/chemistry



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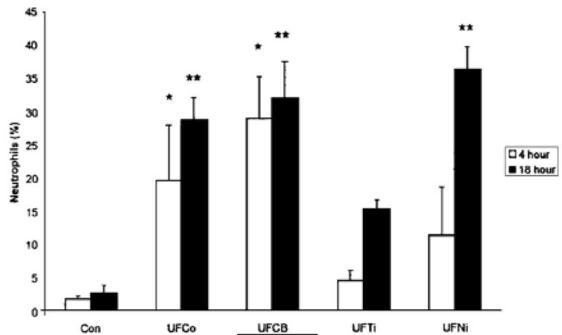
Pulmonary Effects of Nanoparticles

Challenge: Hazard Identification: More Than Size/Surface Area

Pulmonary Inflammation



	Sal	nCo	nCB	nTi	nNi
Dia. nm:		20	14	20	20
SA:		37	254	50	36
Free Radical Activity:		**+++**	**+**	**+/-**	**++**

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1 **Dick et al., *Inhal. Toxicol.* 15, 2003**

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Pulmonary Effects of Nanoparticles

Challenge: Hazard Identification: More Than Size/Surface Area

Nanoscale SiO2: "Toxicity Dependent on Surface Characteristics"

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%

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

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n quartz II = min-u-sil quartz > fine > n quartz I > carbonyl iron

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Warheit et al., *Toxicol. Sci.*, 95, 2007

Pulmonary Effects of Nanoparticles

Challenge: Systemic Effects of Pulmonary Deposited Nanoparticles

SWCNT – Vascular Toxicity

- **Li et al., *EHP*, 115, 2007**
  - -$ApoE^{-/-}$ transgenic, male mice
  - -Purified SWCNT (HiPco)
  - -Oropharyngeal aspiration, 20 µg/mouse, once every 2 weeks for 8 weeks
  - -8 weeks post-exposure
- **Results:**
  - -Inc. plaque size (aorta and BCA) and cellular inflammation in BCA
  - -No evidence of systemic inflammation
  - -Inc. in aortic mitoDNA damage
  - -Evidence of aortic mitochondrial oxidative stress

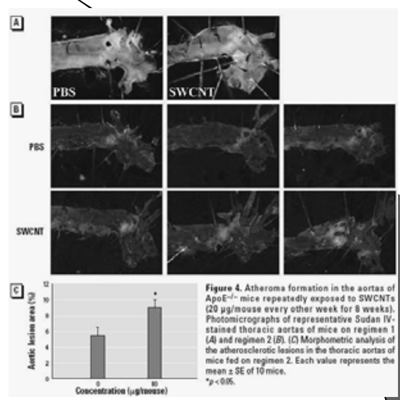
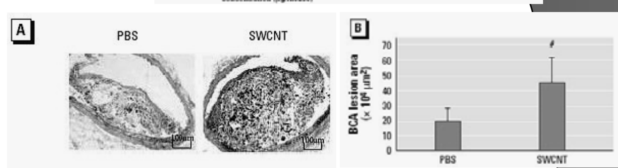


Figure 4. Atheroma formation in the aortas of $ApoE^{-/-}$ mice repeatedly exposed to SWCNTs (20 µg/mouse every other week for 8 weeks). Photomicrographs of representative Sudan IV-stained thoracic aortas of mice on regimen 1 (*A*) and regimen 2 (*B*). (*C*) Morphometric analysis of the atherosclerotic lesions in the thoracic aortas of mice fed on regimen 2. Each value represents the mean ± SE of 10 mice. *$p < 0.05$.

PBS

SWCNT



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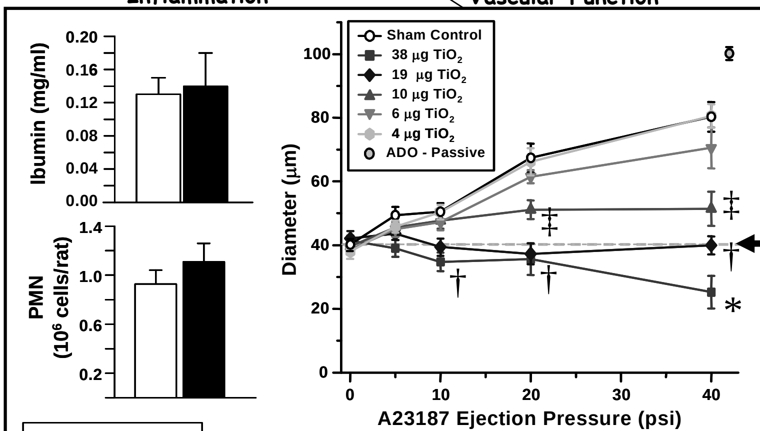
Pulmonary Effects of Nanoparticles

Challenge: Systemic Effects of Pulmonary Deposited Nanoparticles

No Acute Pulmonary Inflammation | **Dose Dependent Alterations in Vascular Function**



In the absence of overt pulmonary inflammation nTiO2 inhalation abolishes or impairs spinotrapezius muscle arteriolar responsiveness to intraluminal A23187 infusion in a dose-dependent manner. *, P<0.05 vs. 19 μg. †, P<0.05 vs. 10 μg. ‡, P<0.05 vs. 6 μg.

Nurkiewicz, et al. 2007, American J. Physiology, Submitted.

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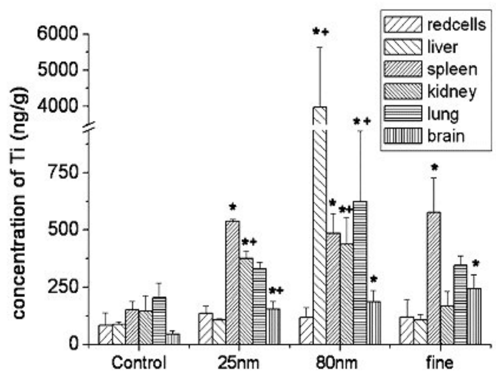
Pulmonary Effects of Nanoparticles

Challenge: Systemic Toxicities Following Oral Exposure to Nanoparticles

- **Wang et al., *Tox. Lett.*, 168:176-185, 2007**
  - **-CD-1(ICR), 19gr, male and female mice**
  - **-oral gavage, 5 gr/kg BW**
  - **-25nm, 80nm, 155nm (fine) TiO_2**
  - **-2 weeks post-exposure**
- **Results:**
  - **-GI uptake and systemic distribution only in female mice (susceptibility)**
  - **-local inflammation in stomach**
  - **-hepatic toxicity (pathology; inc. serum ALT/AST levels)**
  - **-nephrotoxicity (pathology; inc. serum BUN level)**
  - **-myocardial toxicity (inc. serum LDH and HBDH levels)**

Fate of $nTiO_2$ Following Oral Exposure



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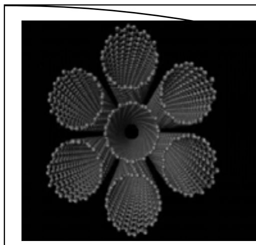
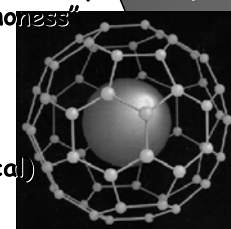


Pulmonary Effects of Nanoparticles: Insight Into Challenges Associated with Nanotechnology Health Risk Assessment

Summary

- **Challenges in Nanoparticle/Nanotechnology Health Risk Assessment**
  - **-Diversity of nanoparticles and their applications**
  - **-Health effects of nanoparticles, their production/applications, and environmental interactions (comprehensive strategy)**
  - **-Nanoparticle deposition, fate, detection in biological systems**

- **Pulmonary Toxicity of Nanoparticles:**
  - **-Size influences deposition and fate (translocation of nanoparticles)**
  - **-Some evidence that toxicity may be unique to "nanoness"**
  - **-Hazard identification is multi-factorial ("more than just size/surface area")**
  - **-Mechanism of injury: oxidative stress (but how; are there other mechanisms)**
  - **-Local vs. systemic toxicities (which are most critical)**
  - **-Host susceptibility factors contribute**



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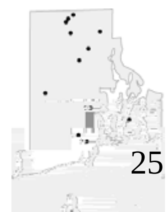
K. Dreher, US EPA, ORD, NHEERL

Nanoparticles: Health Effects

Agnes B. Kane, M.D., Ph.D
Department of Pathology and Laboratory Medicine

Robert H. Hurt, Ph.D.
Division of Engineering

Brown University, Providence, Rhode Island



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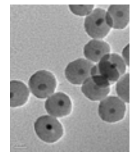
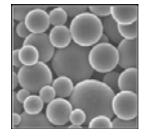
Nanotechnology has Given Rise to an Amazing Variety of New Materials

Equi-axed forms (nanoparticles)
- fullerenes (carbon)
- metallic nanoparticles (e.g. Au, Fe, Ni)
- nanophase ceramics and polymers
- dendrimers
- quantum dots (semiconductor NPs)

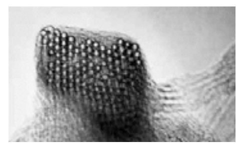


Fe/silica core/shell; Sun, Nurmikko



Carbon nanoparticles

One-dimensional (fibrous) forms
- carbon nanotubes
- nanofibers (carbon, polymer, ceramic)
- nanowires (usually metals)
- nanorods (any chemistry, modest L/D)

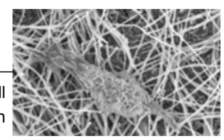


Single-wall nanotube bundle, Thess 1996

Two-dimensional (lamellar) forms

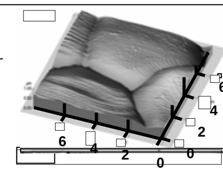
Nanoplatelet graphite, clay, graphene

Nanostructured surfaces

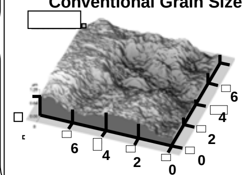


Nanofiber-cell interaction

Nanostructured solids



Conventional Grain Size



Nanoscale Grain Size

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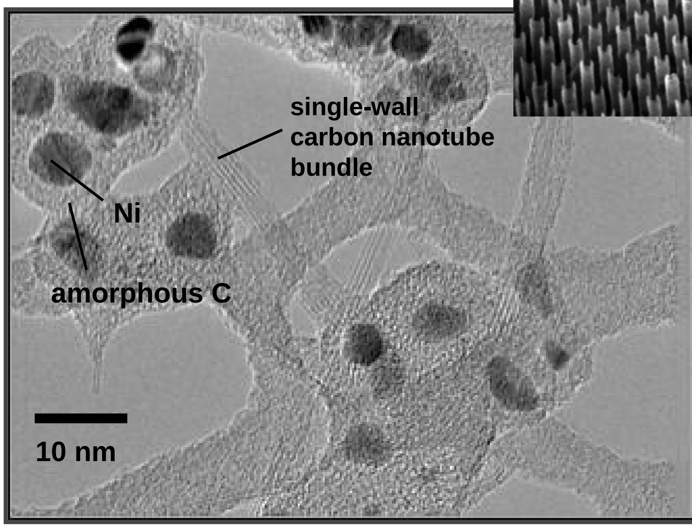
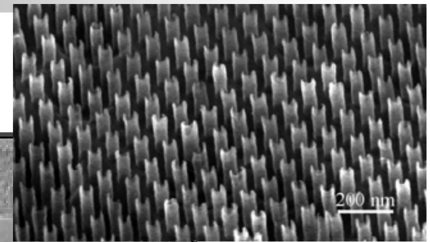
From T.J. Webster

Many Nanomaterial Samples are Complex Mixtures



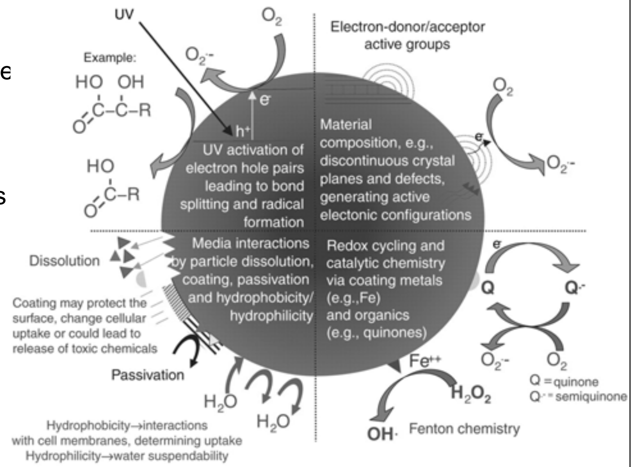


Ideal nanotube structure (J. Xu et al.)

Actual nanotube structure (commercial, as-produced)

Basic nanomaterial properties relevant to toxicity

- Size
  - small: elevated surface are
    and surface activity
- Shape
  - fibrous geometry impedes
    macrophage clearance
- Biopersistence
- Surface chemistry
  - hydrophobicity
  - surface charge
  - redox activity
- Release of chemical toxicants



Surface Reactivity of Nanoparticles
Nel et al. Science 311: 622-627, 2006

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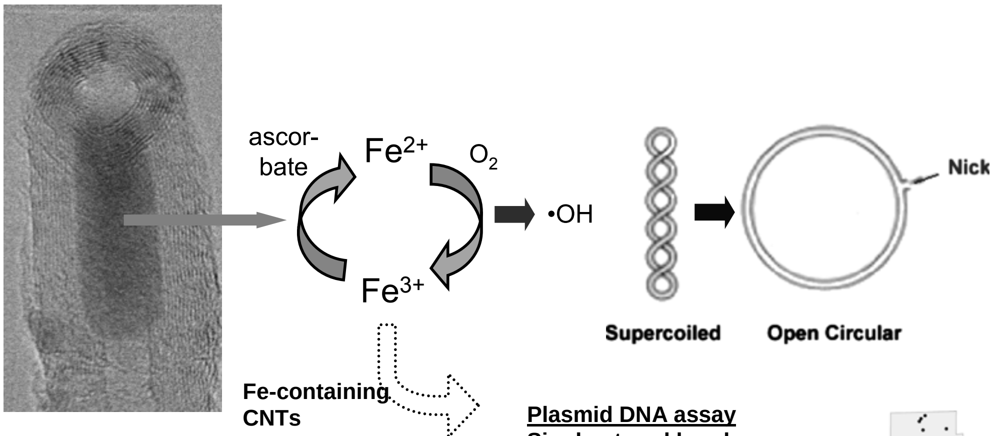
Carbon nanotubes can be redox activity through release of bioavailable iron



<u>**Plasmid DNA assay**</u>
Single-strand break
Induces uncoiling event
detectable by
gel electrophoresis

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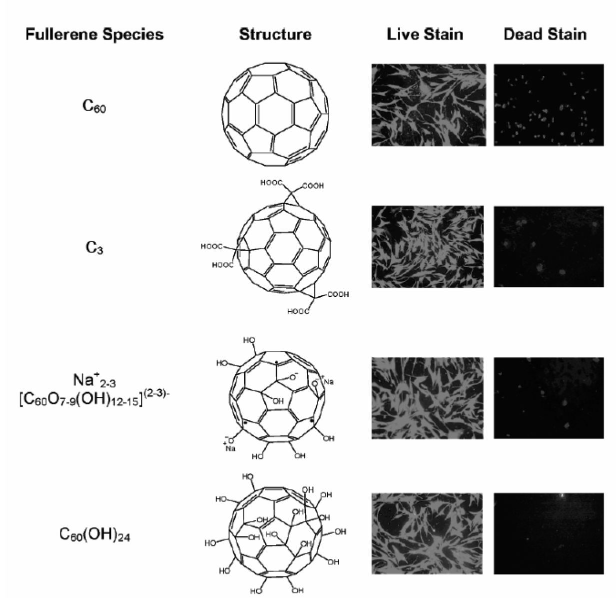
Cytotoxicity of Fullerenes Depends on Surface State



Sayes et al.
NANO LETTERS
2004 Vol. 4, No. 10
1881-1887

Carbon material hydrophobicity varies with synthesis, processing and functionalization
[1] "Controlling Water Contact Angle on Carbon from 5 to 167 Degrees" [Yan et al., 2006]

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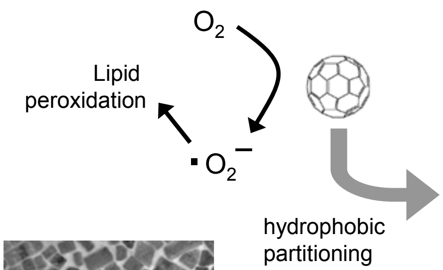
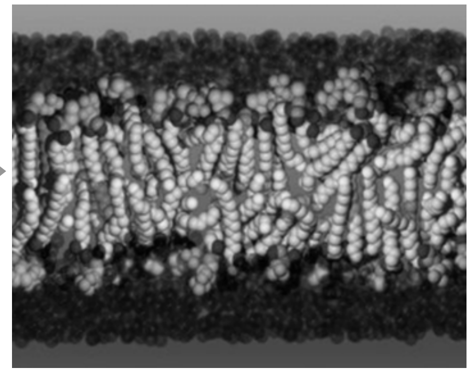
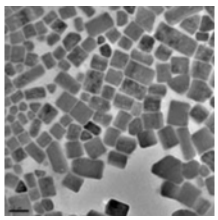
Possible Mechanisms of Fullerene Toxicity

based on Sayes et al., 2004, Oberdorster, 2004



Simulated lipid bilayer structure





Unsubstituted fullerene aggregates in aqueous media to form “nano-C60”

Postulated mechanism:
Hydrophobic attachment to / incorporation in cell membranes with redox catalysis of lipid peroxidation

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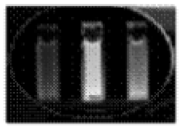
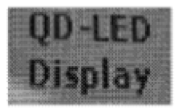
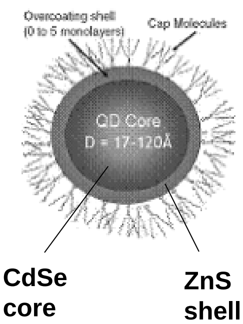
Some important nanomaterials contain known chemical toxicants imbedded in core/shell structures

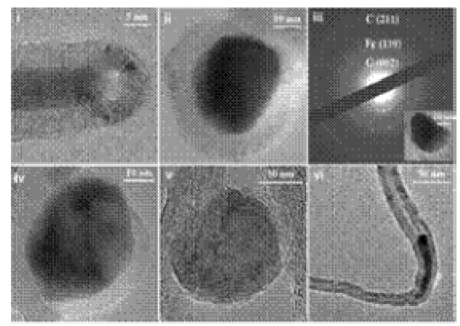




Quantum dot fluorescence, example LED display application, and core/shell structure



Imbedded metal structures in carbon nanotubes



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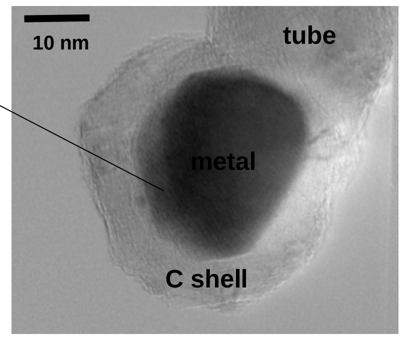
Catalyst Residues in Carbon Nanotubes

- Catalytic growth methods:
  - now dominant for synthesis of multiwall nanotubes (esp. large scale)
  - only route for single-wall nanotube synthesis
- Most common elements in CNT catalyst formulations are Fe, Ni, Y, Co, Mo
- Ultrafine metals pose documented inhalation health risks depending on form, exposure route, dose

Bioavailability?
(the key issue)

- Do metals contribute to CNT toxicity? How can we assay for and manage CNT metals effects?



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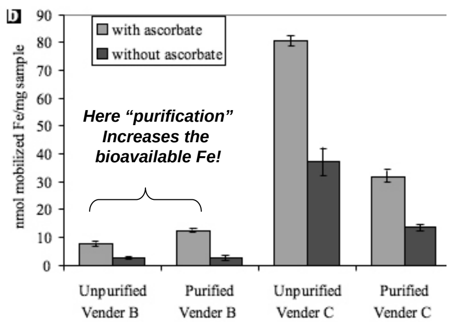
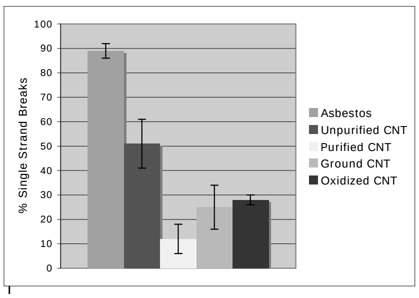
Iron Bioavailability and Redox Activity of Diverse Carbon Nanotube Samples

From Guo et al., Chemistry of Materials 2007



Fe mobilization



DNA single-strand breaks

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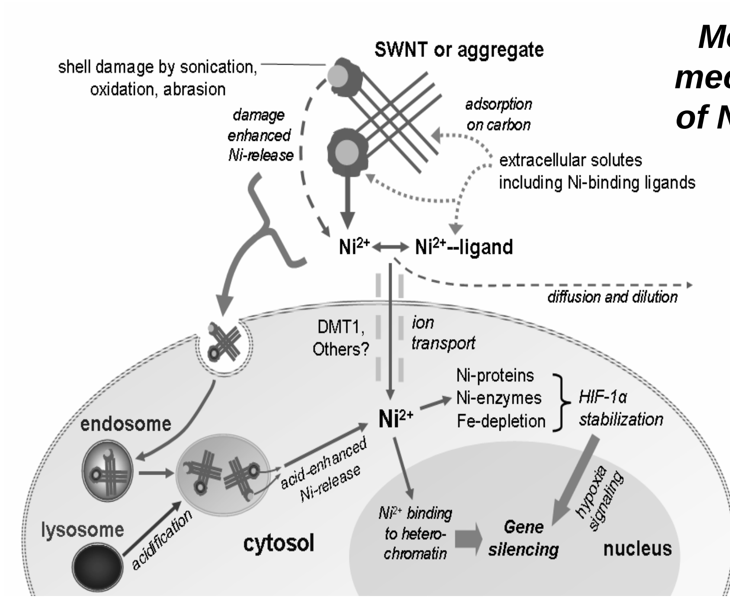
Molecular mechanisms of Ni toxicity



![BROWN]

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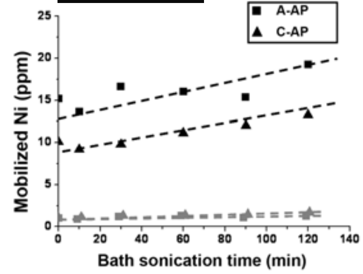
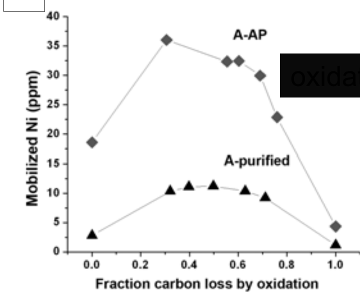
Environmental and Processing Stresses Affect Metal Bioavailability and Toxicity

[Liu et al, Advanced Materials, in press]

[redacted]



[redacted]



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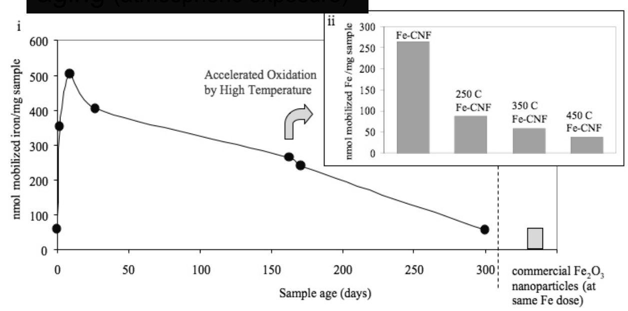

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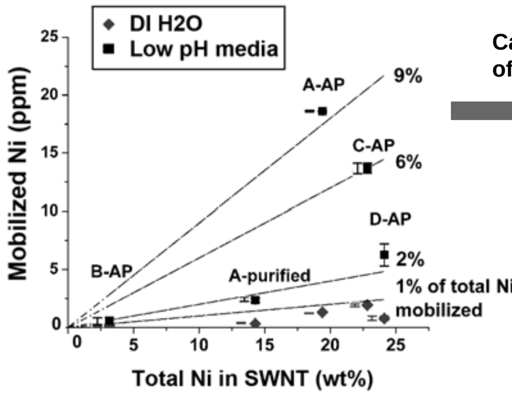
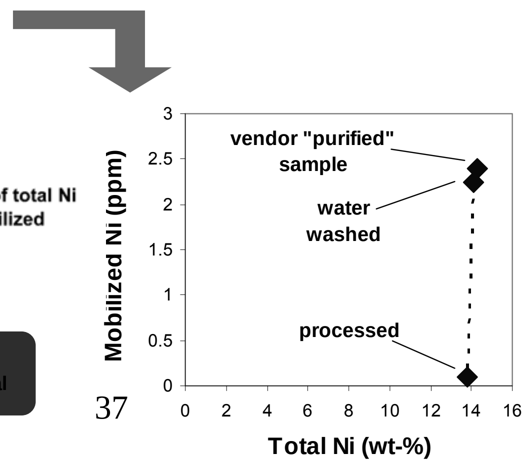
Toward Carbon Nanotube Detoxification



The bioavailable metal represents only from 0.5% to 9% of the total metal

Can we target the *bioavailable* fraction of total metal for removal?



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Toxicology of Engineered Nanomaterials

[Oberdörster et al., Environ. Health Persp. 113:823-839, 2005]

EXPOSURE ⟶ DOSE ⟶ RESPONSE

EXPOSURE

Source? ***air***
water
food
diagnostic or medical device

Route of uptake? ***inhalation***
ingestion
skin
injection
implantation

DOSE

Dose metric? ***mass***
number
surface area

which organ?
which cell?
persistence at this site?

RESPONSE

portal of entry?
systemic distribution?
remote effects?

desirable effects?
diagnostic
therapeutic

toxicity?
oxidative stress
immune function
acute vs. chronic

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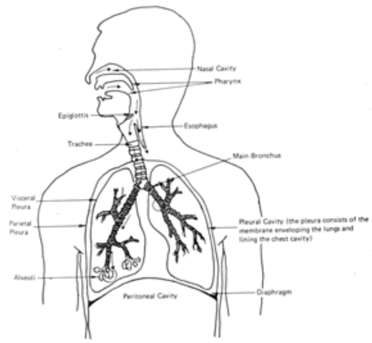
Are Nanomaterials the Next Asbestos Fibers?

History of Asbestos-Related Diseases

Becklake, Am. Rev. Resp. Dis. 114:187-227,1976

Disease	Suspected	Established Causal Association
Asbestosis	1900	1930
Lung Cancer	1930	1955
Mesothelioma	1940	1965
Cancer of Larynx	1955	2006
Nanodiseases	2001	????



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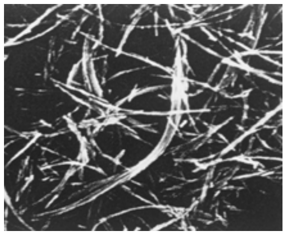
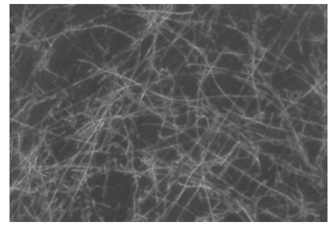
Properties of Fibers Relevant for Biologic Activity



chrysotile asbestos

Fiber dimensions
Chemical composition
Durability
Surface reactivity

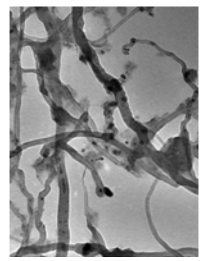


carbon nanofibers

Catalyst Precursors for Carbon Nanotube Production

Moisala et al., 2003

Iron sulfate hydrate
Iron ammonium sulfate
Iron, nickel, or cobalt nitrate
Iron chloride
Iron oxides



Lin Guo, Engineering graduate student

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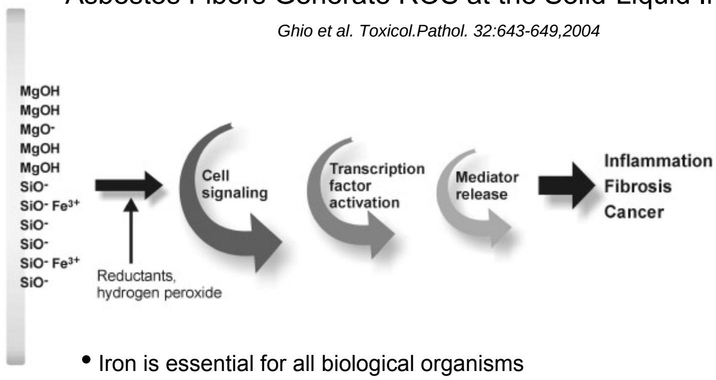
Asbestos Fibers Generate ROS at the Solid-Liquid Interface

Ghio et al. Toxicol.Pathol. 32:643-649,2004



- Iron is essential for all biological organisms
- Iron is tightly bound to extracellular proteins (transferrrin, lactoferrin) or to intracellular proteins (enzymes, ferritin)
- Asbestos fibers contain redox active iron linked with toxicity
- *Can redox-active iron be mobilized from carbon nanotubes?*

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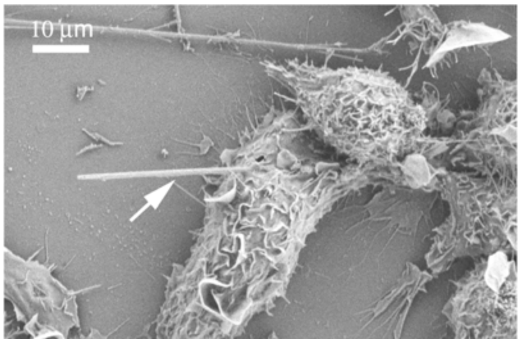
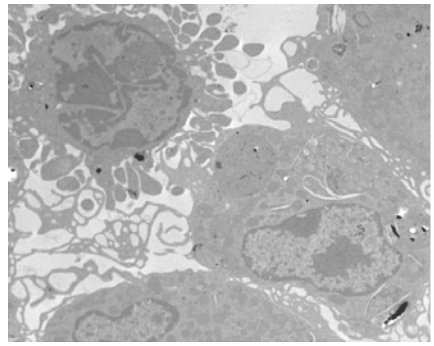


Frustrated phagocytosis of an asbestos fiber by a macrophage



Induction of cell death (apoptosis)

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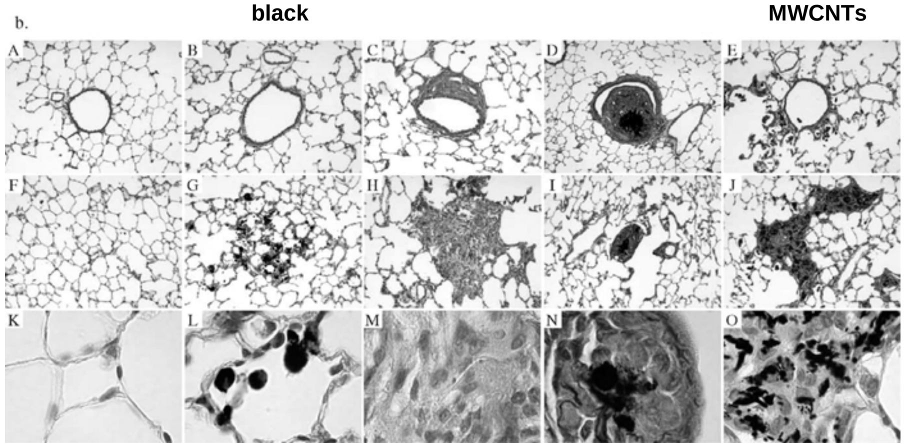
Lung Toxicity of Multiwall Carbon Nanotubes

Muller et al. Toxicol. Appl. Pharmacol. 207: 221-231, 2005

Saline | **Carbon black** | **Asbestos** | **MWCNTs** | **Ground MWCNTs**



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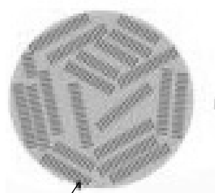
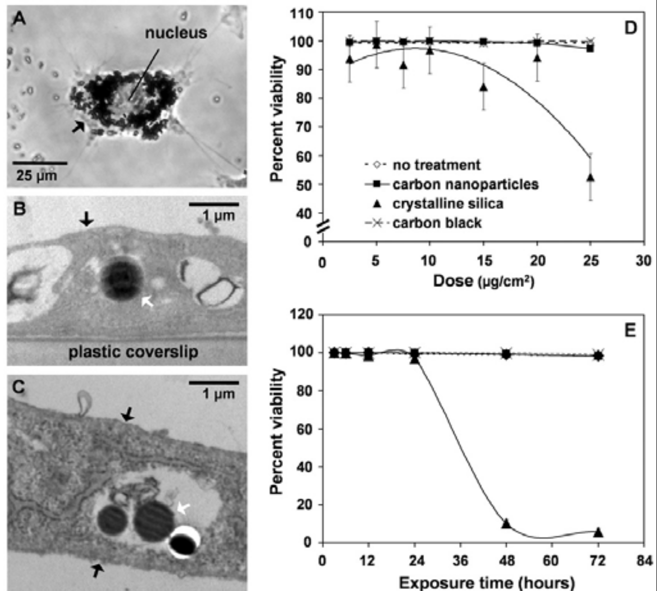
"Supramolecular" carbon nanoparticles are taken up by mesothelial cells and are non-cytotoxic

(Brown work, Yan et al., 2006)



Outward facing graphene layers provide active sites for functionalization



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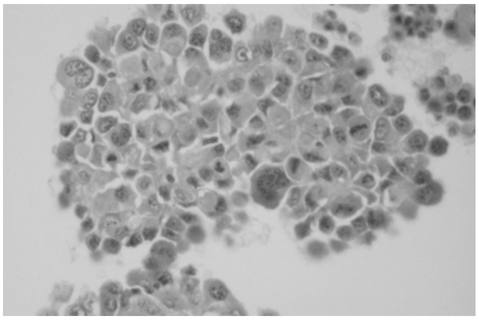
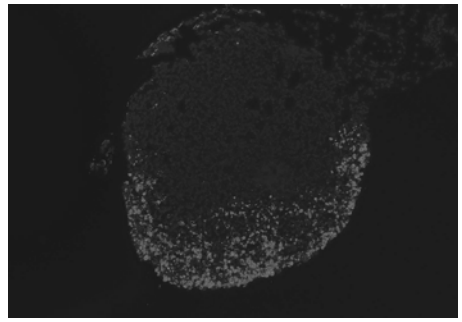
•**Penetration of nanoparticles into solid tumor masses**





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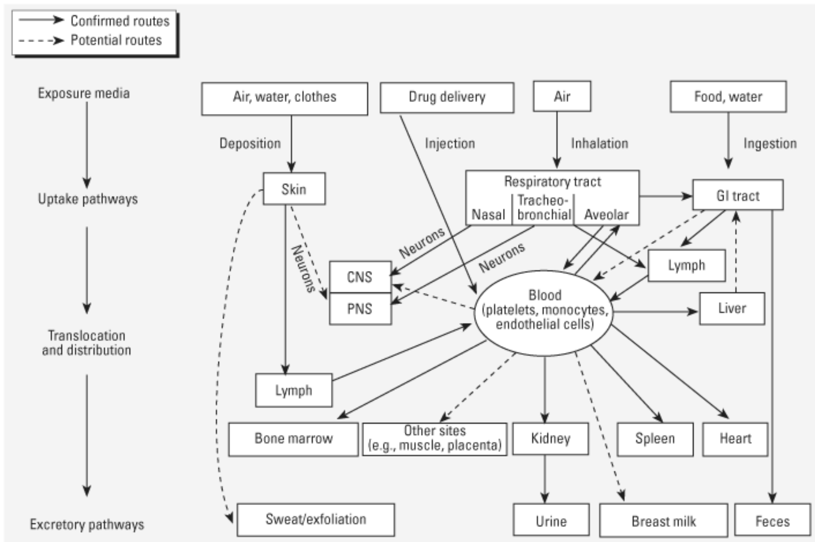
Systemic Distribution of Nanoparticles

Oberdörster et al., Environ. Health Perspect. 113: 823-839, 2005



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POTENTIAL CHRONIC TOXICITY OF NANOMATERIALS

NEL ET AL. SCIENCE 311: 622-627, 2006

TISSUE TARGET	DISEASE
Macrophages and inflammatory cells	granulomas chronic inflammation fibrosis or scarring
Lungs	cancer, mesothelioma
Blood vessels	stroke, heart attack
Immune system	autoimmune disease leukemia, lymphoma
Nervous system, brain	heart arrhythmia brain injury

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Occupational Carcinogens

Agent	Industry	Target Site
Arsenic	Glass, metal, pesticide	Lung, Skin
Asbestos	Construction	Lung, Pleura, Larynx
Benzene	Chemical	Leukemia
Beryllium	Aerospace	Lung
Cadmium	Dyes, batteries	Lung, Prostate, Kidney
Chromium (VI)	Metal plating, welding	Lung, Nasal Sinus
Nickel	Metallurgy, alloys, catalyst	Lung Nasal Sinus
Crystalline silica	Mining, glass, pottery	Lung
Sulphuric acid mists	Metallurgy	Larynx

P. Boffetta, Epidemiology of environmental and occupational cancer, Oncogene 23: 6392-6403, 2004.

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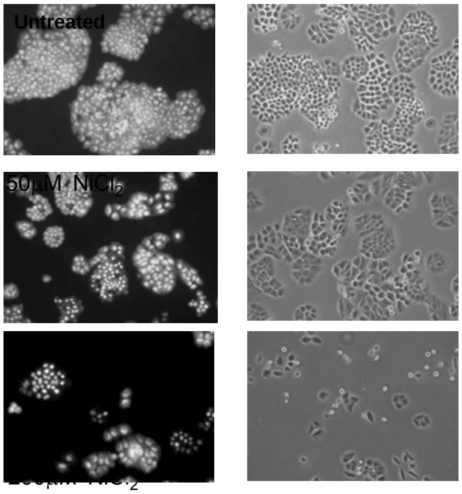
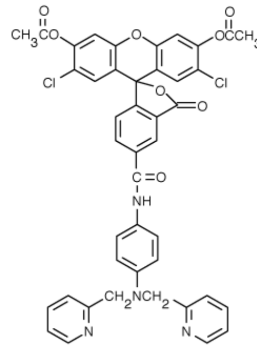
Toxicity of $NiCl_2$ Using Human Lung Epithelial Cells In Vitro

Syto-10/Ethidium Homodimer Viability Assay – 48 hours (100x)

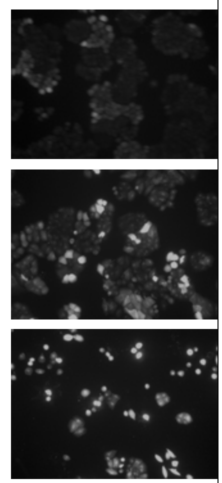
Phase Contrast Microscopy – 48 hours (100x)





Newport Green DCF

Newport Green Fluorescence – 48 hours (200x)



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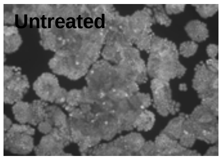
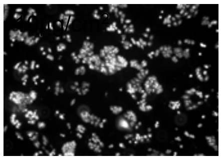
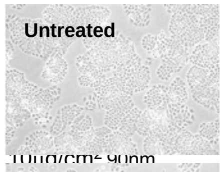
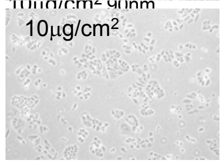
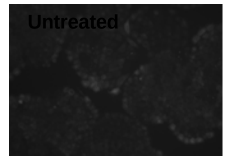
Toxicity of Metallic Nickel Nanoparticles

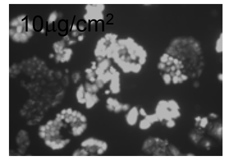






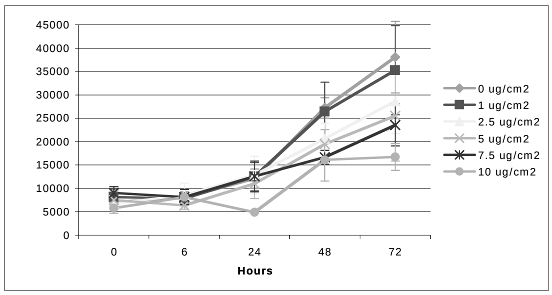






Syto-10/Ethidium Homodimer Viability Assay
- 72 hours (100x)

Newport Green fluorescence
- 72 hours- (200x)



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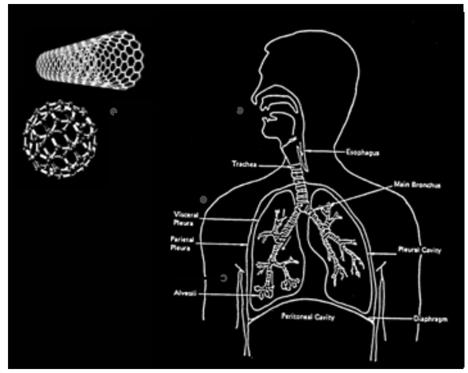
The Opportunity



Most nanomaterials are:

- fabricated, not natural
- developmental, not commercial

Fabrication/purification processes greatly affect key toxicity variables:

- size, shape, surface chemistry
- metals content and location

Ultimate Goal: **Understand material structure / toxicity relationships to guide development of “green” nanomaterials**

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BROWN

Risk Assessment of Nanomaterials

Steve Roberts

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University of Florida

Nanotoxicology at the University of Florida

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Overview of Issues

The premise for nanotechnology is that nanoscale materials have new, beneficial properties:

- unique physical-chemical and material properties
- new biological properties that will be useful for diagnostics and therapeutics

If biological properties change as materials move to nanoscale, then maybe our knowledge of the toxicological properties of these materials no longer applies.

- kinds of effects produced
- doses at which effects occur

Concerns for Risks from Nanotechnology

Examples of calls to slow or halt nanotechnology development

"Until more is known about environmental impacts of nanoparticles and nanotubes, we recommend that the release of manufactured nanoparticles and nanotubes into the environment be avoided as far as possible."

Nanoscience and Nanotechnologies: Opportunities and Uncertainties, 2004

"At this stage, we know practically nothing about the potential cumulative impact of human-made nano-scale particles on human health and the environment. Given the concerns raised over nanoparticle contamination in living organisms, ETC Group proposes that governments declare an immediate moratorium on commercial production of new nanomaterials and launch a transparent global process for evaluating the socio-economic, health and environmental implications of the technology."

The Big Down, AtomTech: Technologies Converging at the Nano-scale, 2003

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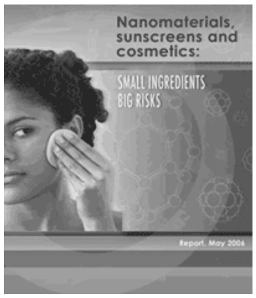
Filling the Void



from Friends of the Earth, 2004

Sense of risk is conveyed not by affirmative evidence of hazard, but rather by the absence of evidence for safety.

Federal Funding for EHS Research

Table 6
Budget for Environmental, Health, and Safety R&D, 2006–2008
(dollars in millions)

	2006 Actual	2007 Estimate	2008 Request
NSF	21.0	25.7	28.8
DOD	1.0	1.0	1.0
DOE	0.5	0.0	3.0
DHHS (NIH)	5.2	5.4	5.7
DOC (NIST)	2.4	2.8	5.8
NASA	0.0	0.0	0.0
EPA	3.7	7.9	9.6
USDA (CSREES)	0.1	0.1	0.1
DHHS (NIOSH)	3.8	4.9	4.6
USDA (FS)	0.0	0.0	0.0
DHS	0.0	0.0	0.0
DOJ	0.0	0.0	0.0
DOT (FHWA)	0.0	0.0	0.0
TOTAL	37.7	47.8	58.6

From *Supplement to the President's FY 2008 Budget, National Nanotechnology Initiative*

Risk Assessment Steps

Hazard Identification

- Determination whether a particular substance is causally linked to particular health effects.

Exposure Assessment

- Determination of the extent of exposure before or after application of regulatory controls.

Dose-response assessment

- Determination of the relationship between the magnitude of exposure and the probability of occurrence of the health effects in question.

Risk Characterization

- Description of the nature and often the magnitude of human risk, including attendant uncertainty.

Adapted from *Risk Assessment in the Federal Government: Managing the Process, NRC 1983*

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Challenges in Studying Toxicity

Time to complete the studies - A battery of studies to satisfy regulatory concerns about safety can take years to complete.

Prioritizing nanomaterials for study - Field is advancing quickly; nanomaterials of interest are rapidly replaced by newer nanomaterials.

Consistency of materials - Manufacturing techniques for many nanomaterials are still being worked out; poor quality control.

Relevant exposures - Almost no studies available showing actual exposure conditions.

Amount of materials required for testing - New nanomaterials are often available only in small quantities and are very expensive.

More Challenges …

Characterizing the test material - Both chemical and physical properties of nanoscale materials can change with time, handling, and in biological environments.

Characterizing the dose - There is uncertainty as to whether doses should be expressed in terms of mass, surface area, or particle concentration.

Measurement of nanomaterials in tissues - Detection and quantification of nanostructures in tissues is difficult. High resolution microscopy (e.g., transmission electron microscopy) is often required.

Hazard Identification

What kinds of health effects do nanoscale materials produce?

- Are the effects the same as produced by the same material in conventional scale?


MATERIAL SAFETY DATA SHEET

OSHA - Meets 29 CFR 1910.1200 Standards

HMIS HAZARD RATINGS			
HEALTH	1	0 = INSIGNIFICANT	3 = HIGH
FLAMMABILITY	4	1 = SLIGHT	4 = EXTREME
REACTIVITY	2	2 = MODERATE	

TRANSPORTATION INFORMATION			
PROPER SHIPPING NAME:	Aluminum powder, uncoated		
HAZARD CLASS / PKG GRP:	4.3 / III	REF:	49 CFR 173.151, .213, .244
IDENTIFICATION NUMBER:	UN 1396	LABEL:	Dangerous When Wet

HEALTH HAZARDS (ACUTE AND CHRONIC): The toxicological properties and health hazards associated with this material as a very fine powder have not been investigated. However, it is assumed this product presents the same health hazards as the product in standard powder form. Use appropriate procedures and protective equipment to prevent opportunities for direct contact with the skin or eyes and to prevent inhalation.

CARCINOGENICITY NTP? No IARC MONOGRAPHS? No OSHA REGULATED? No

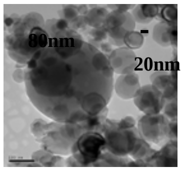
MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE: Preexisting skin, eye, or respiratory disorders may become aggravated through prolonged exposure.

Hazard ID Points

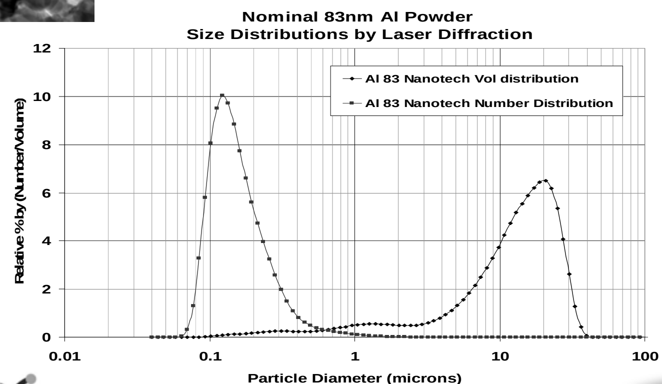
Changes in properties in moving to nanoscale:

1) could lead to fundamentally different biological effects and/or profound changes in absorption or distribution of the material (compared with conventional scale).
2) can be different for different materials.
3) can result from changes in size, shape, and/or surface characteristics.
   - This greatly complicates hazard identification, particularly since the factors influencing toxicity for each material are, at this point, largely unknown.
   - Adequate characterization of materials used in toxicity tests is extremely important.
   - Characterization should include the material as administered, not just as received.

Size Matters - But what size is it?



Median Diameter (by Volume)		14.4 microns
Median Diameter (by Number)		151 nm
Specific Surface Area	$25.7 m^2/g$	80 nm (equivalent)



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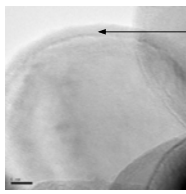
Surface Properties

For aluminum particles, cytotoxicity appears to be determined by the oxide coat.

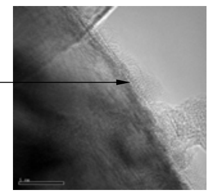
- Aluminum oxide passivates the surface, decreasing reactivity and toxicity.
- The stability of the aluminum oxide coat is a function of particle size; smaller particles have less stable coats and are more cytotoxic.



2.5 nm oxide layer

2.5 nm oxide layer

80 nm aluminum



- The oxide coat varies depending upon length of exposure to air or water.
- Results can vary depending not only upon size, but also “aging” of material.

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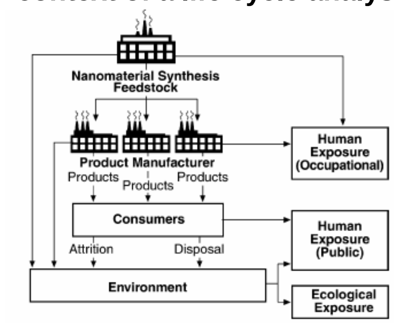
Exposure Assessment

Potential exposure scenarios should be considered in the context of a life cycle analysis



From Tsuji et al., Tox. Sci. (2006), v. 89(1), pp 42-50.

Exposure Assessment Issues

Nanoscale materials pose special issues for each of the three primary routes of exposure:

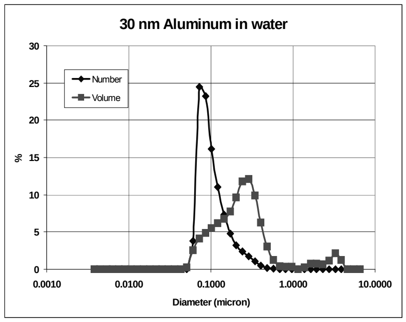
- inhalation
- dermal absorption
- ingestion

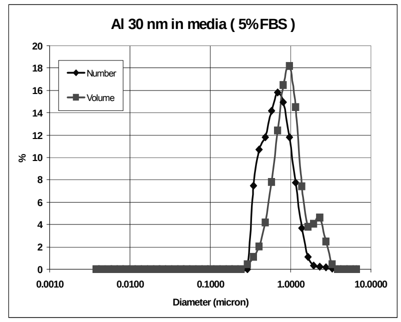
Toxicity studies should be conducted using the same nanomaterial forms that exist under actual exposure conditions

- for example, extent of aggregation, surface properties
- understanding of environmental fate is critical

Altered Properties During Exposure

Comparison of size distribution of aluminum nanoparticles in water versus culture media with 5% fetal bovine albumin (FBS)





Courtesy of M. Palazuelos, UF

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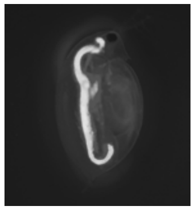
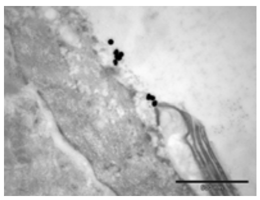
Detection



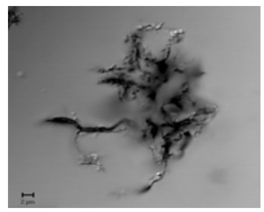
Quantum dots in GI tract of *Daphnia*
Courtesy of J. Griffitt, UF

Detection of structurally-intact nanomaterials can be very challenging, particularly in tissues.

The most straightforward way to observe and measure nanoparticles in tissues is with microscopy, but microscopic techniques are technically demanding.



Colloidal gold on mouse skin
Courtesy of S. Wasdo, UF



Fluorescence tagged SWCNTs
Courtesy of R. Mercer, NIOSH

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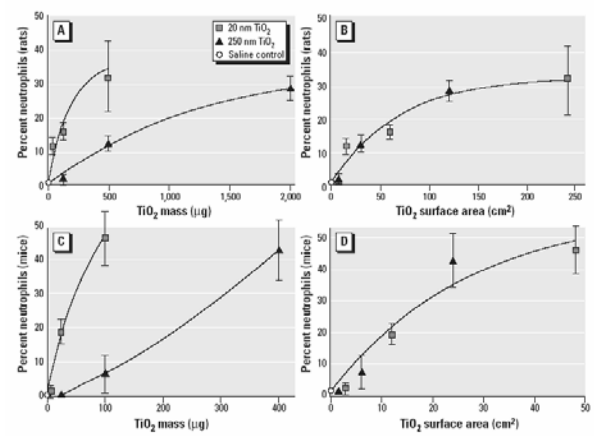
Dose-Response Assessment

What is the correct dose metric?



From Oberdoster et al., EHP (2005), v. 113(7), pp. 823-839

Recent Initiatives

Prioritization of Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials

- Nanotechnology Environmental and Health Implications Working Group, National Science and Technology Council
- Available on www.nano.gov; deadline for public comments is 09/17/07

Concept Paper for the Nanoscale Materials Stewardship Program under TSCA

- Available at epa.gov/oppt/nano/nmspfr.htm; deadline for public comment is 09/10/07

FDA Task Force Report on Nanotechnology, July 2007

- Available at www.fda.gov/nanotechnology/taskforce/report2007.html

Approaches to Safe Nanotechnology

- Available at www.cdc.gov/niosh/topics/nanotech/safenano/

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

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Conclusions

The existing risk assessment paradigm is suitable for evaluation of nanomaterials.

Data to support risk assessments is generally lacking. Impediments to developing data include:

- Regulatory ambiguity on testing requirements
- Limited availability of funding for testing and research
- Technical challenges in conducting toxicity studies
- Data developed by industry is often unavailable

We currently have a risk assessment "void" being filled with speculation.

Federal agencies are moving forward to clarify the regulatory status of nanomaterials and sponsor environmental health and safety research.

Credits

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Kevin Powers

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Center for Environmental & Human Toxicology, UF

Scott Wasdo

Joe Griffitt

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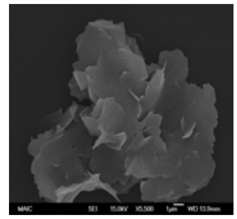
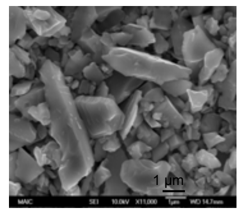


aluminum flakes



Quartz Min-U-Sil

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