Effects of Nanomaterials on Human Health

Animal Models

Anna A. Shvedova, PhD, DSc, FATS

Pathology & Physiology Research Branch/NIOSH/CDC,
Department of Physiology and Pharmacology,
School of Medicine, WVU, Morgantown, WV

Nano.gov
National Nanotechnology Initiative
http://nano.gov/

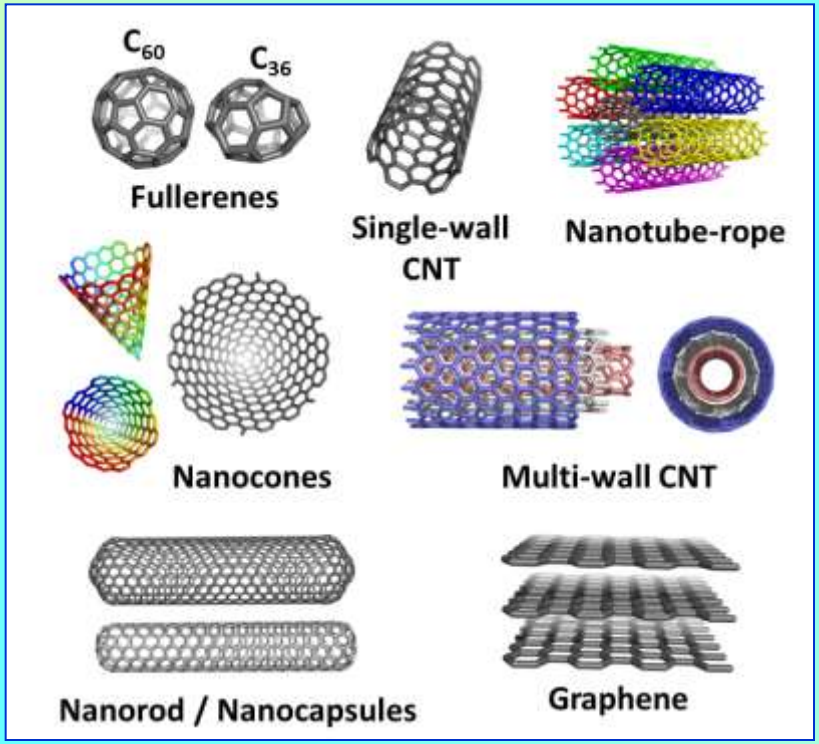
NSF, Arlington, VA
December 2-3, 2013

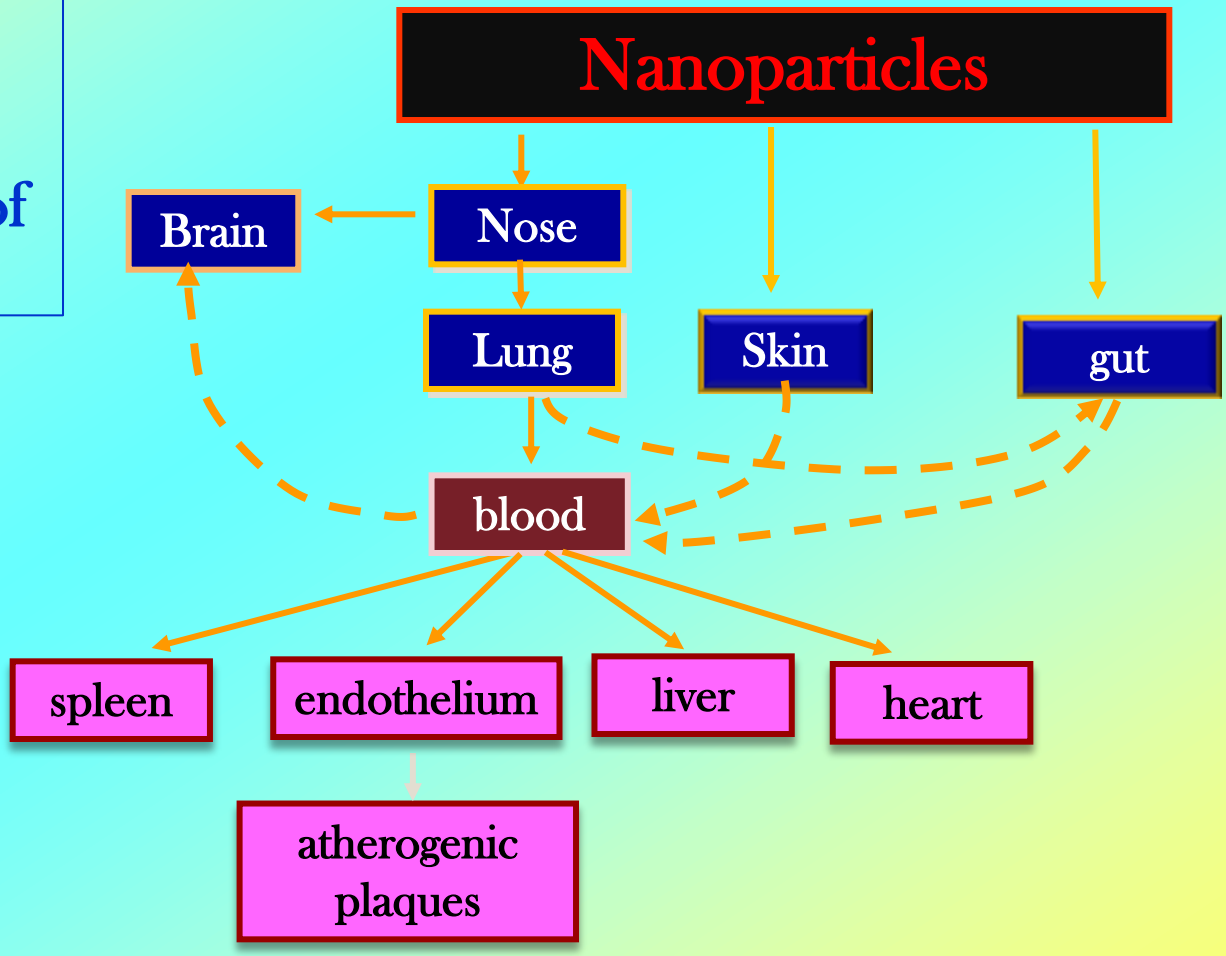
Special concerns associated with nanoparticles.

Analogy to fine particle pollution:

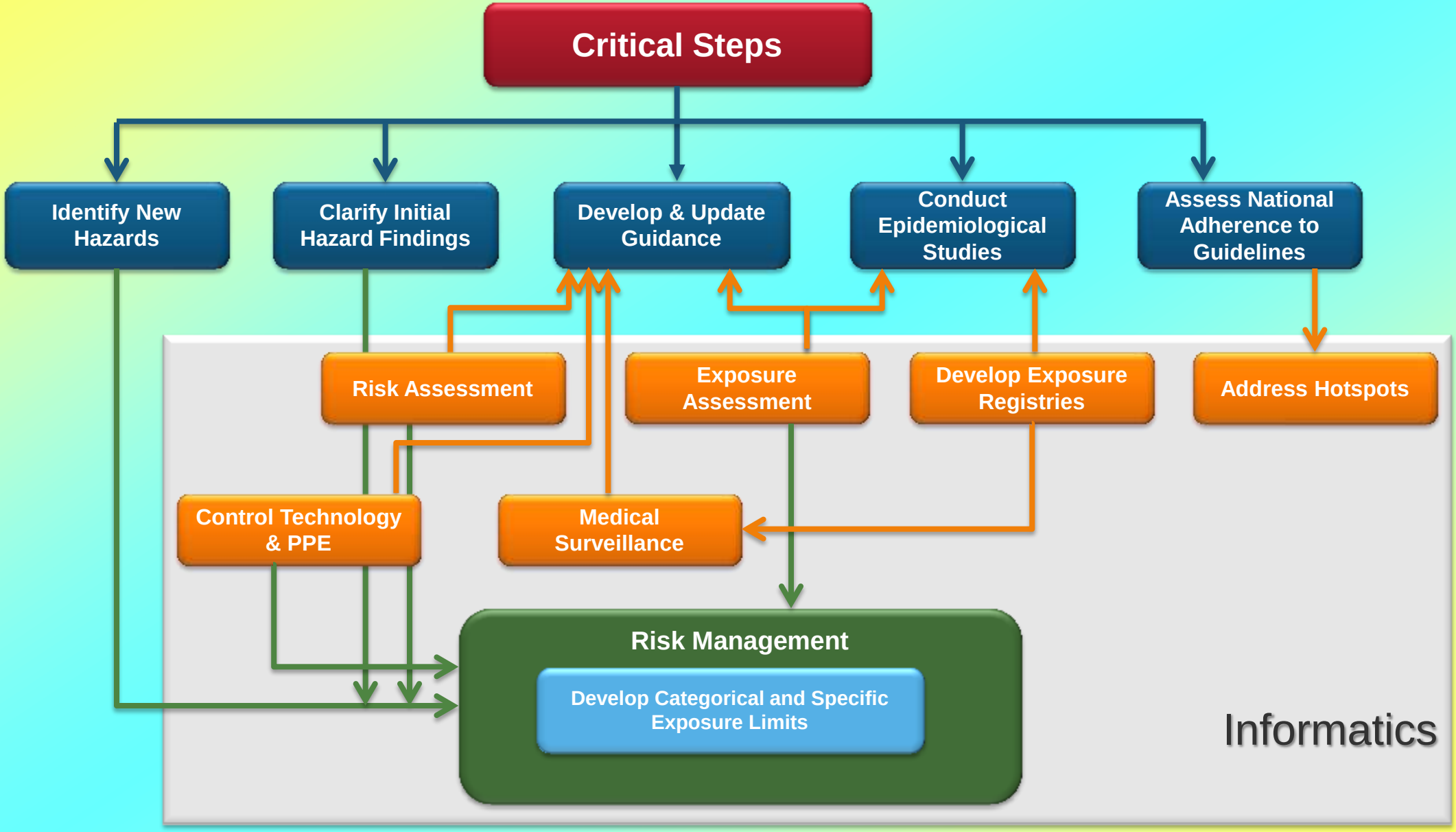
Ability to move around the body

- Possible shared mechanisms of toxicity





Current Focus of NIOSH Nanomaterial Research



NIOSH NTRC Plan 2013-2016

Nanotechnology : Risk Management Process

Hazard Identification:
Determine possible adverse effects

↓

Hazard Characterization:
Dose response, time course, mechanisms

↓

Exposure Assessment:
Airborne levels generated by nanotech workplace processes

↓

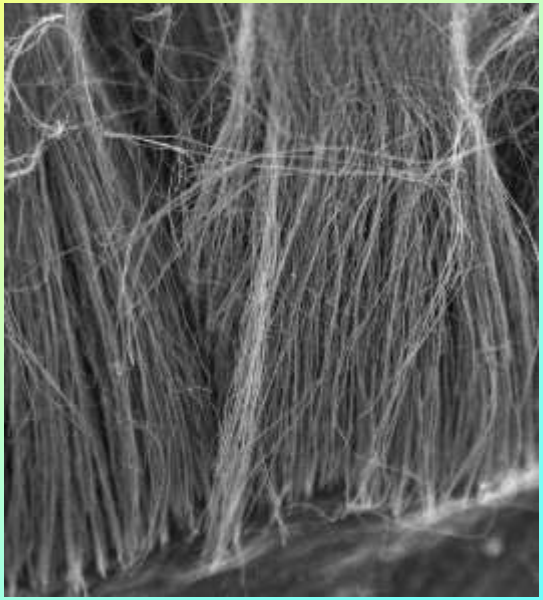
Risk Assessment:
Modeling to relate human response to animal reactions

↓

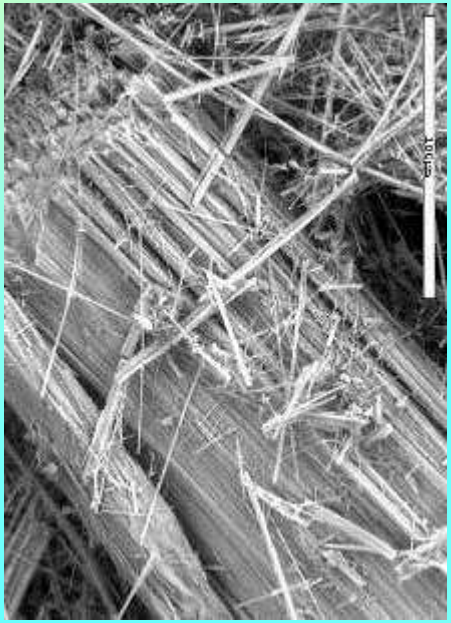
Risk Management:
Development of controls and safe work practices

Example Scenario: CNTs

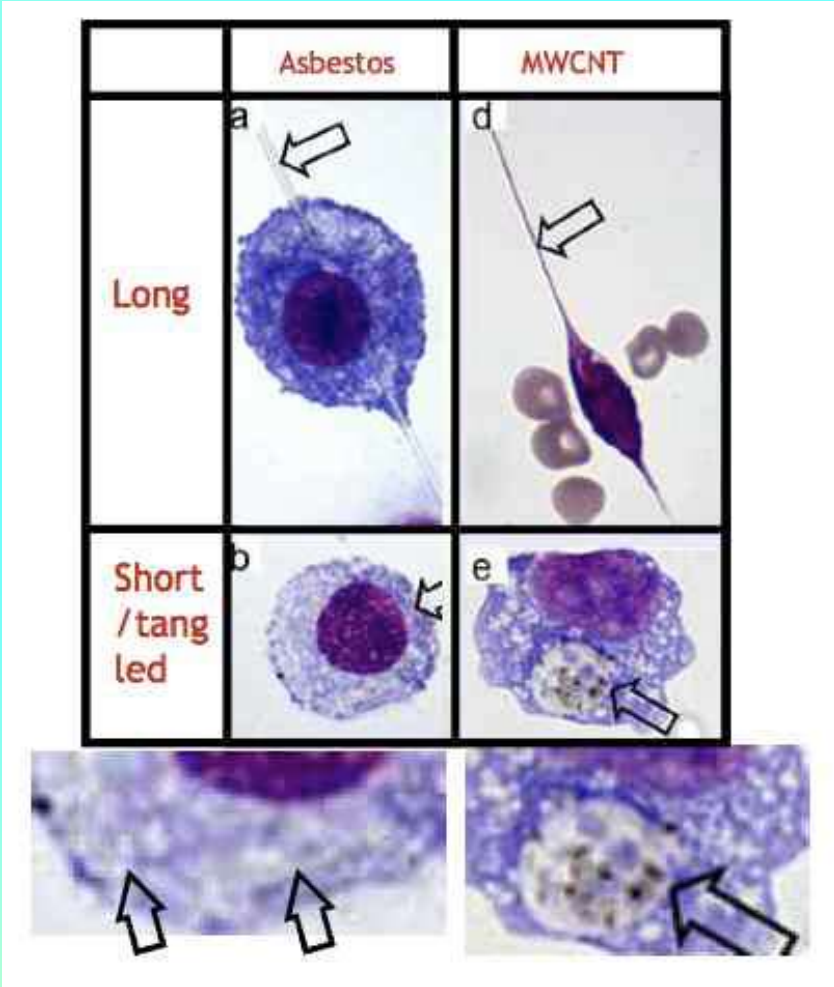
Why be concerned about exposure to nanotubes?



Multi-walled carbon nanotubes

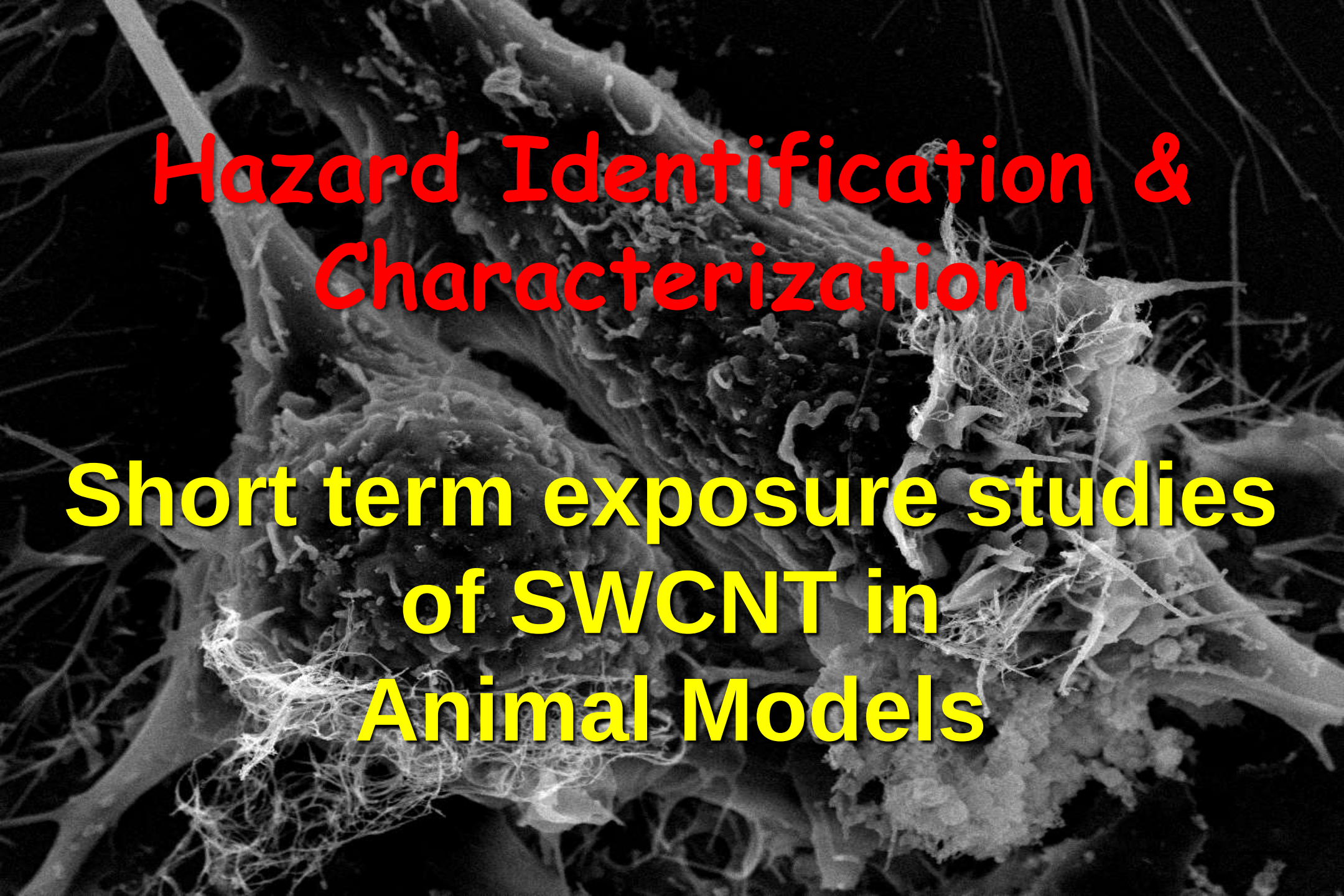


Asbestos

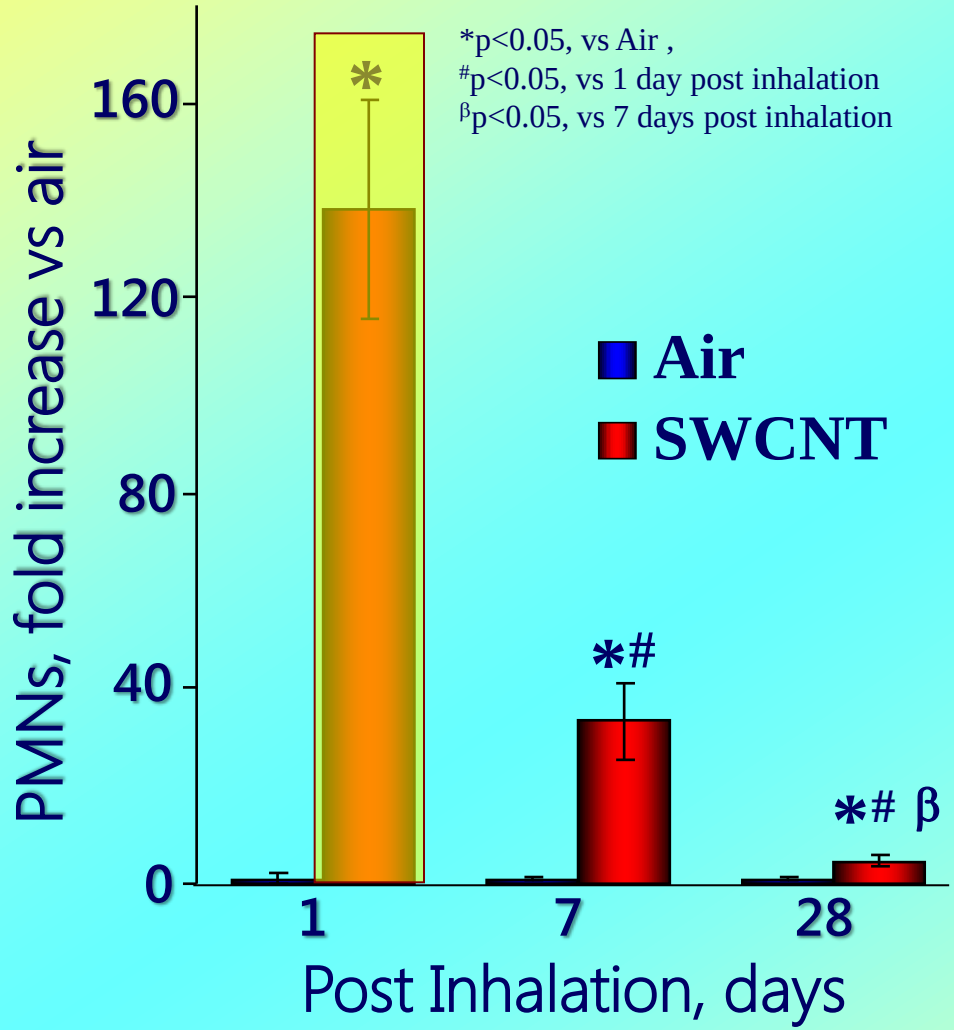


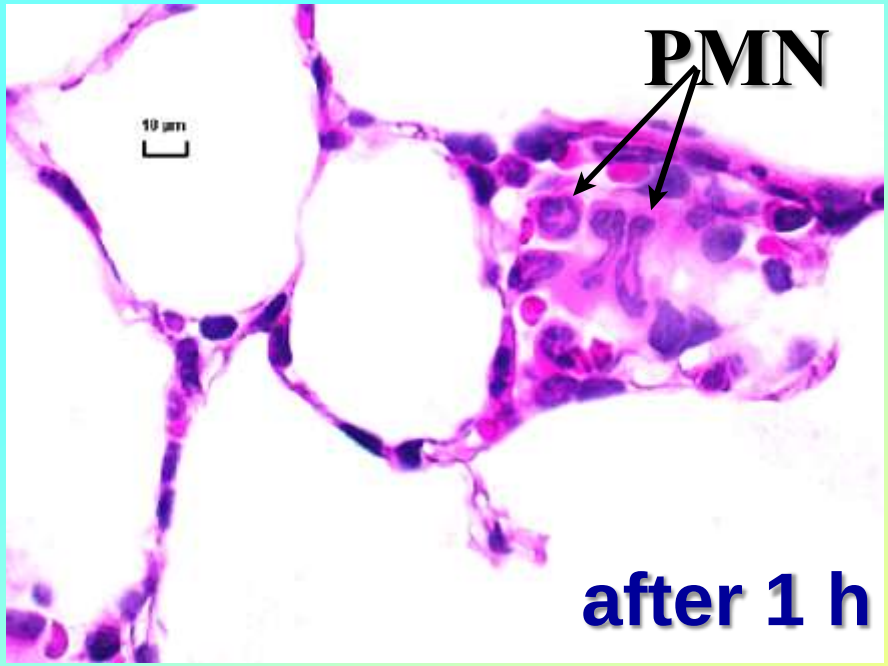
Frustrated phagocytosis induced by asbestos and multi-walled carbon nanotubes.
(Poland CA et. al. Nature Nanotechnology 3, 423 - 428 (2008))

Biopersistence of long asbestos fibers can lead to inflammation, granuloma formation, fibrosis, and cancer.


Hazard Identification & Characterization
Short term exposure studies of SWCNT in Animal Models

Inflammatory Response in Mouse Lung upon Exposure to SWCNT

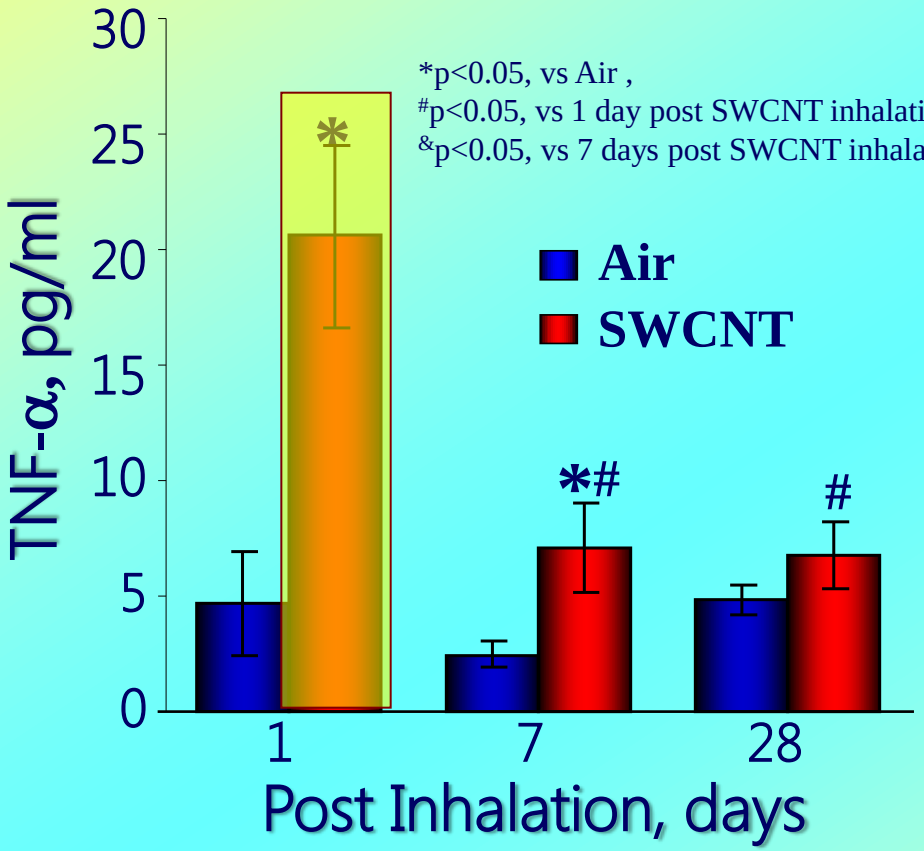


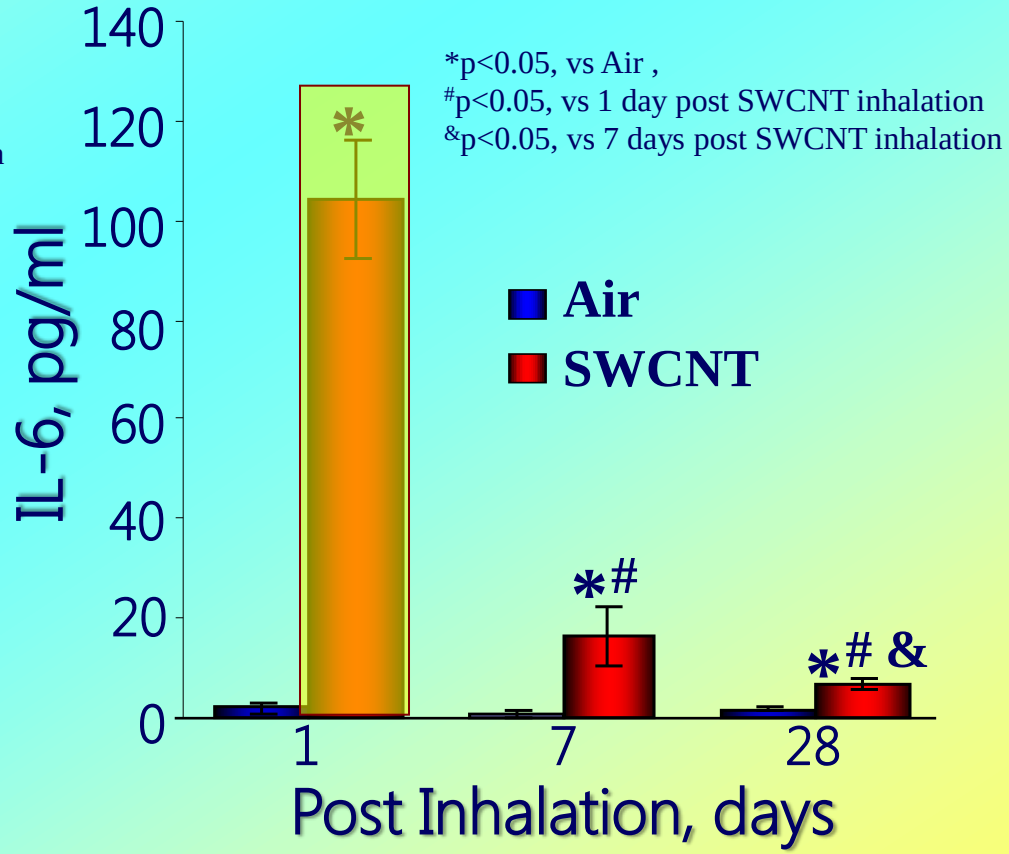


Courtesy of R. Mercer

Accumulation of Pro-Inflammatory Cytokines in BAL Fluids of C57BL/6 Mice after Inhalation with SWCNT

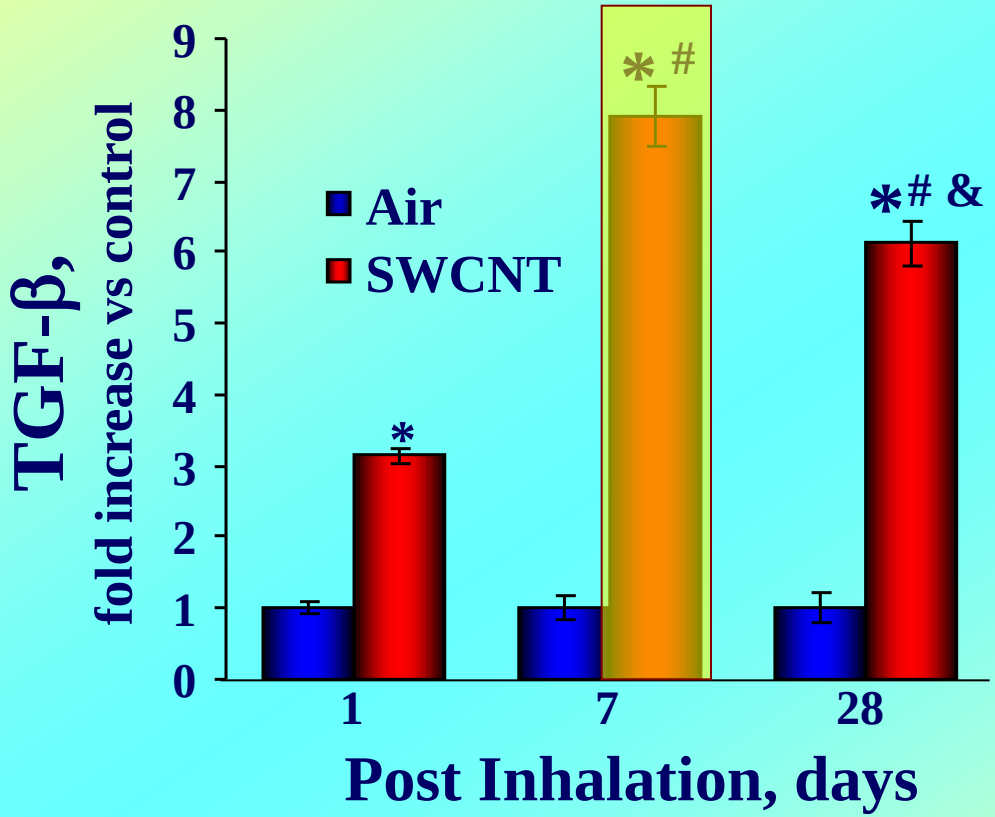
(5 mg/m^3, 5 h a day, 4 days)





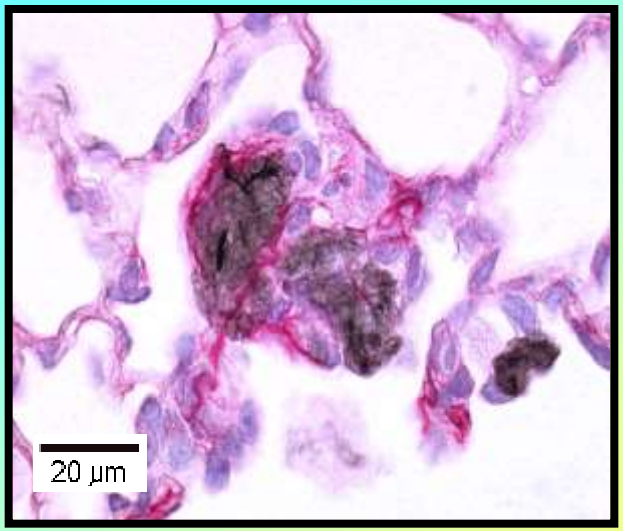
Levels of Pro-Fibrotic TGF-β in BAL Fluid from C57BL/6 Mice Exposed to SWCNT

(5 mg/m³, 5 h a day, 4 days)



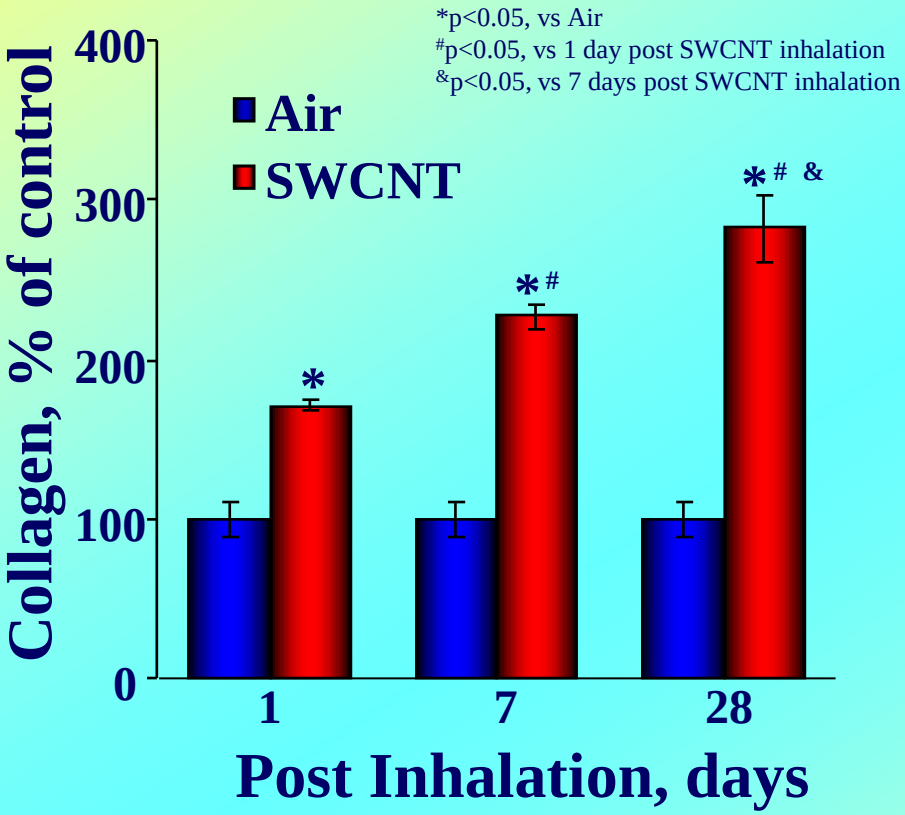
Collagen Fibers Surrounded/ Interdigitated SWCNT Deposits (Granuloma)

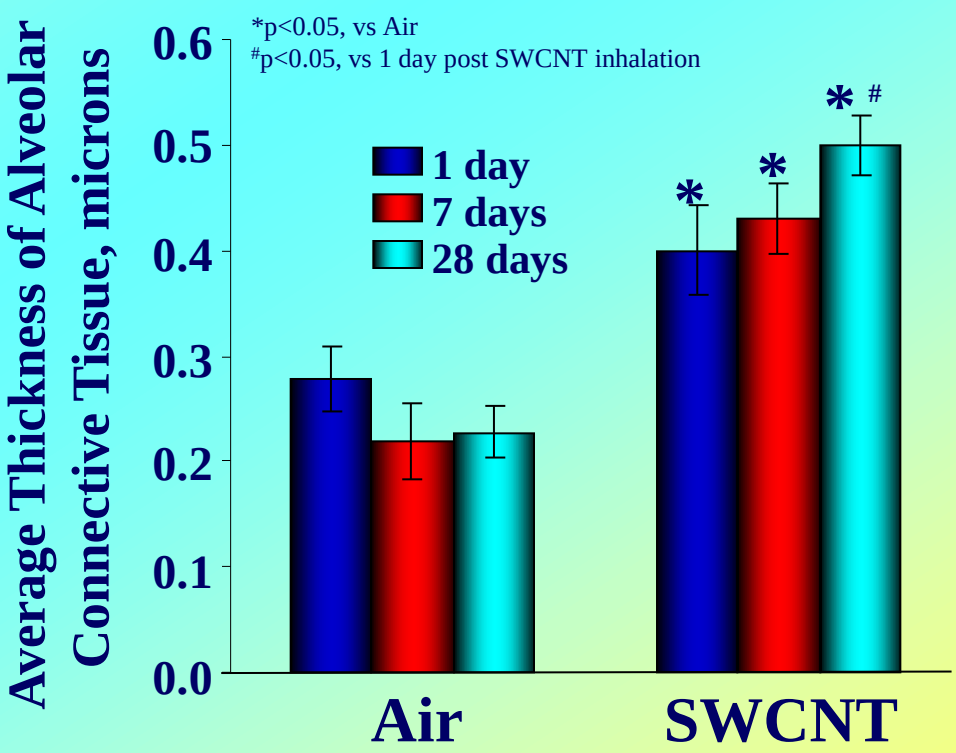
7 days Post Exposure



Light Micrograph of Sirius Red Stained Lung Section of C57BL/6 Mouse

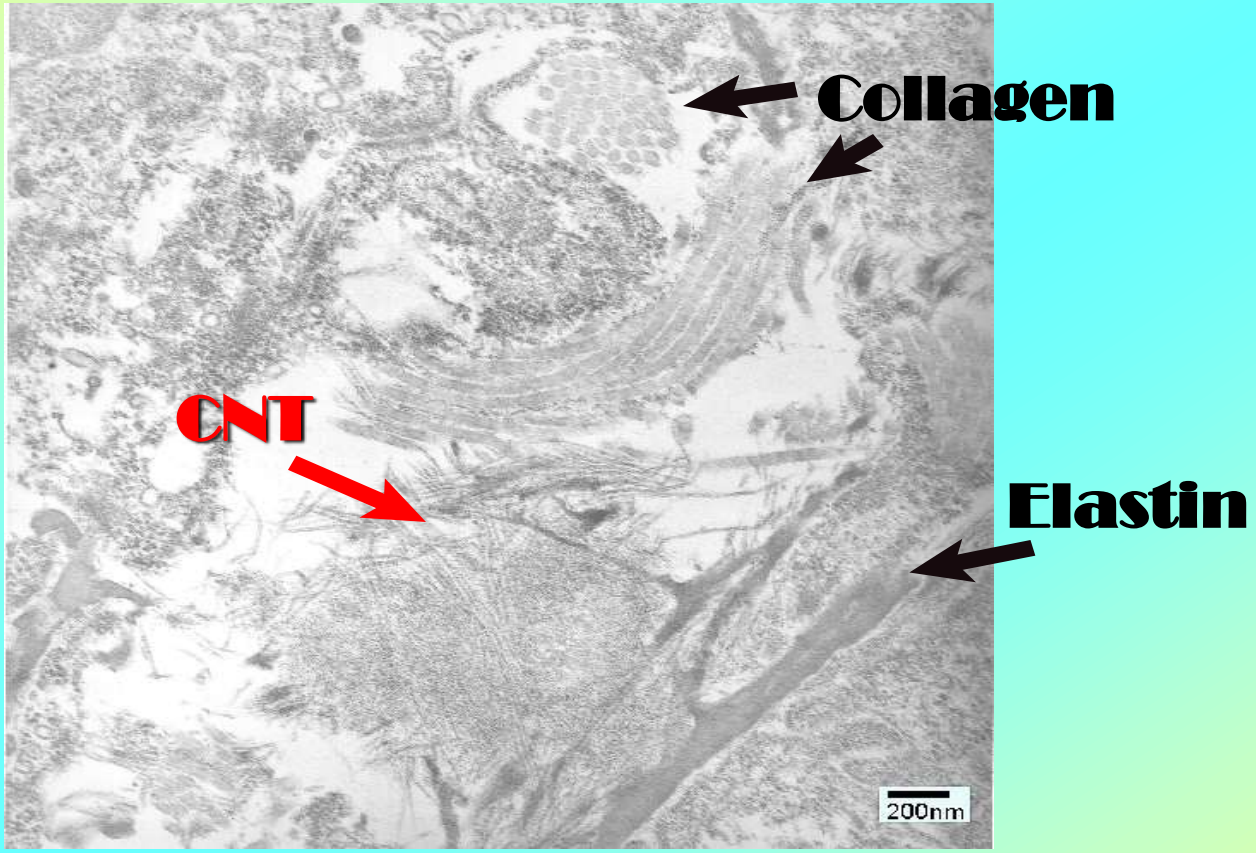
Collagen Accumulation and Morphometric Changes in the Lungs of C57BL/6 Mice after Inhalation with SWCNT



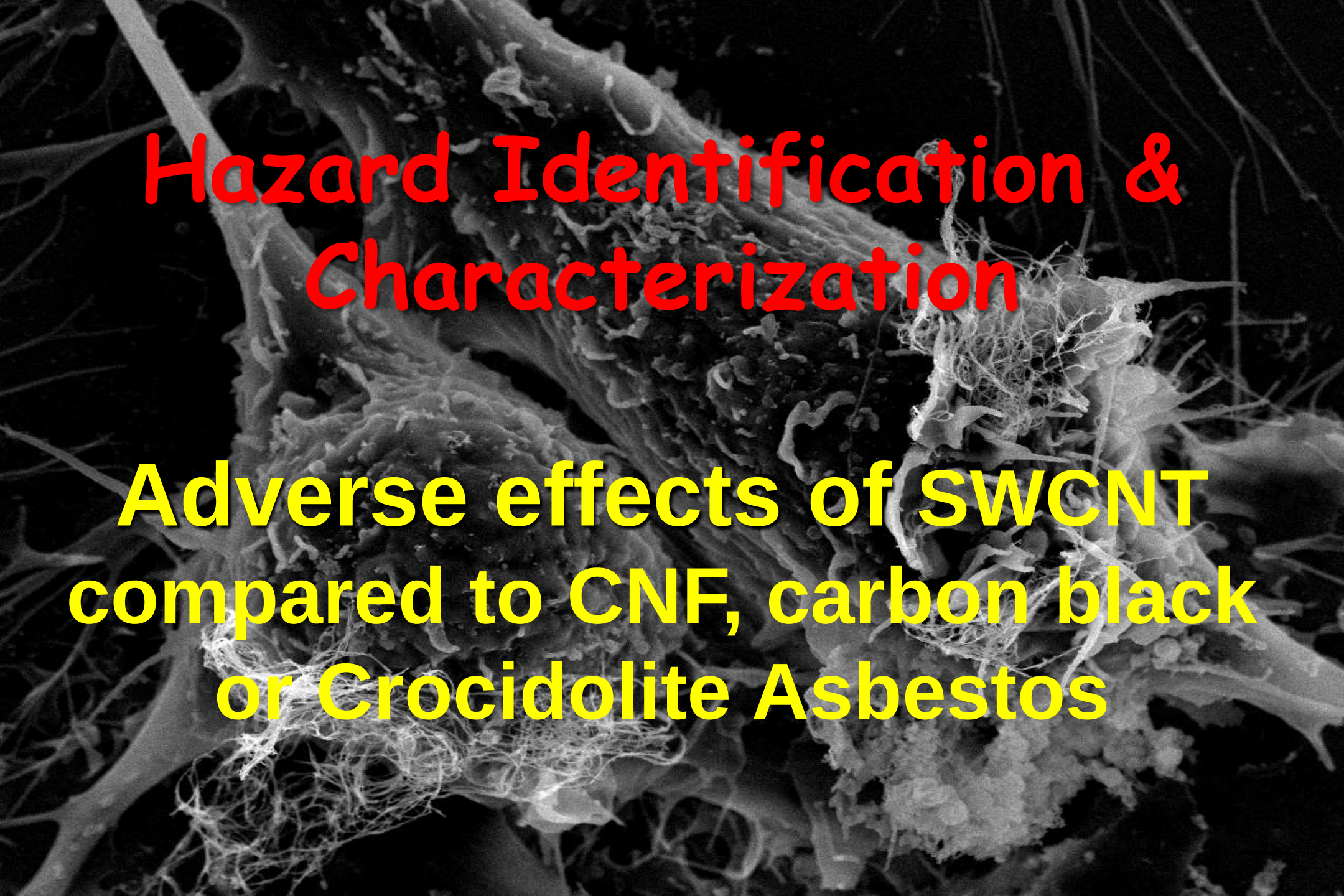


Airborne Exposure : 5 mg/m^3, 5 h/day, 4 days

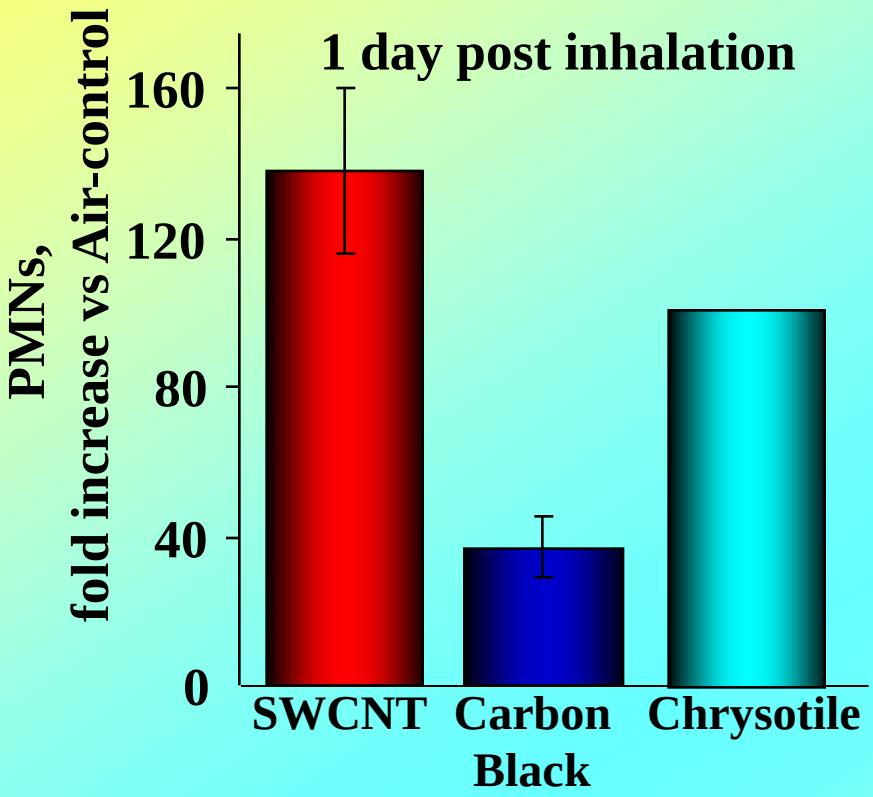
Carbon Nanotubes in Interstitial Space

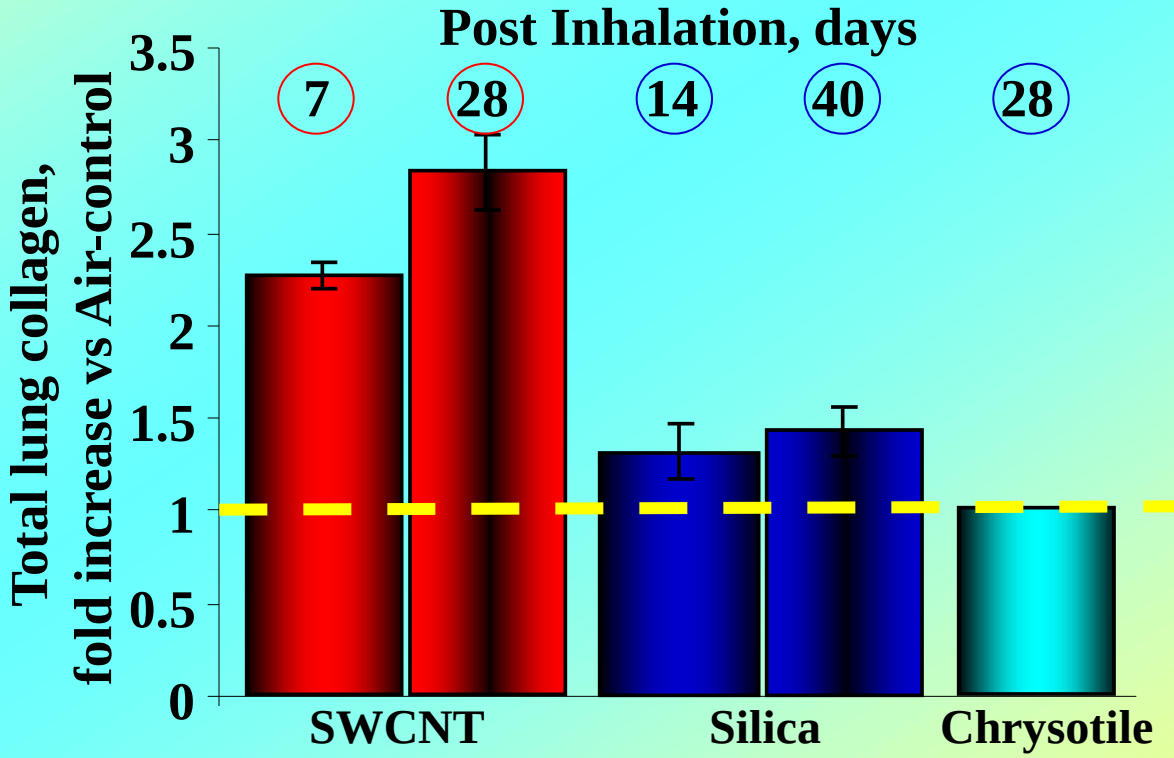


TEM of carbon nanotubes in interstitial space. Micrograph shows carbon nanotubes intermixed with normal connective tissue matrix of the lungs


Hazard Identification & Characterization
Adverse effects of SWCNT compared to CNF, carbon black or Crocidolite Asbestos

Pulmonary Inflammation and Fibrosis in Mice after Inhalation with SWCNT, Carbon Black, Silica or Asbestos





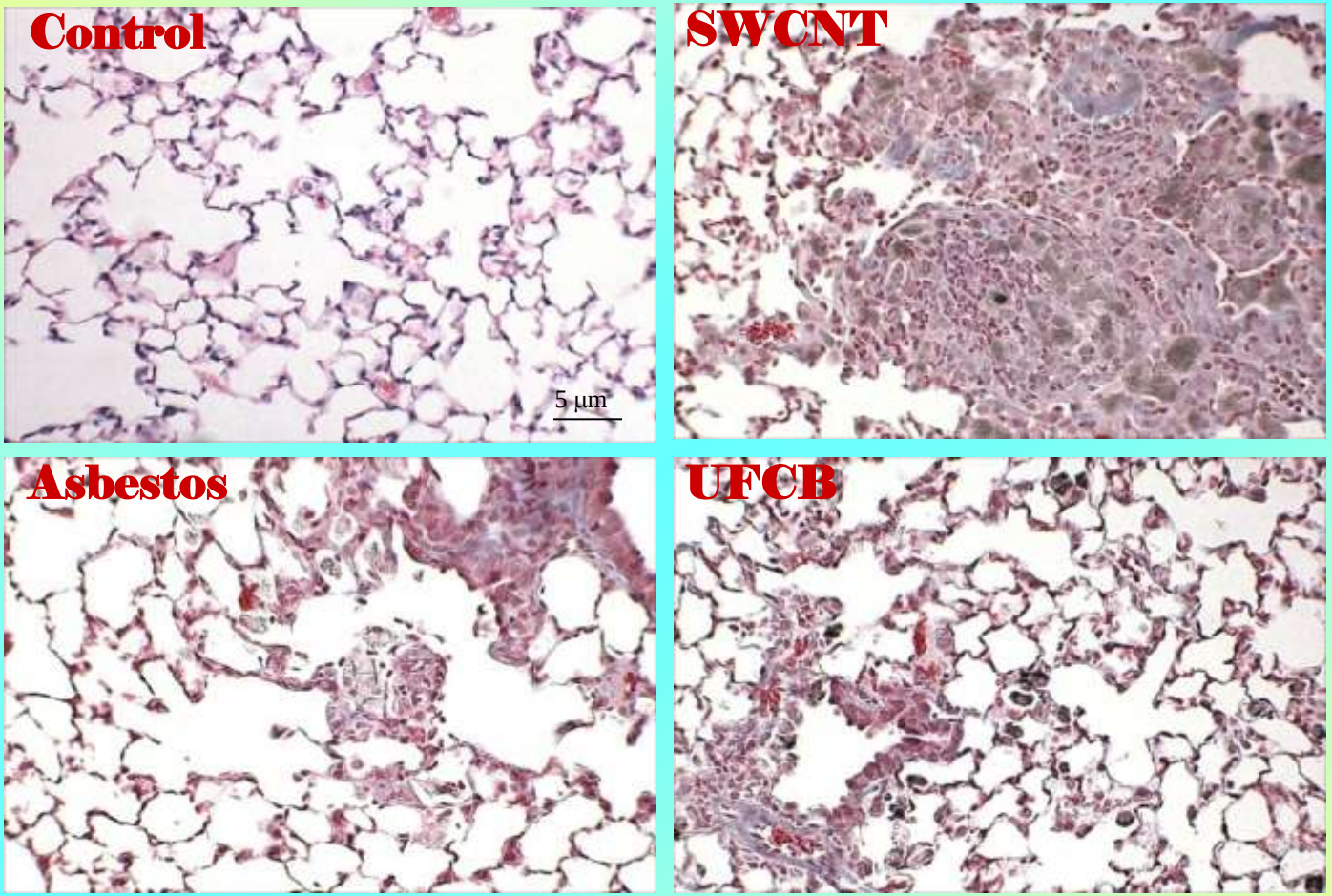
SWCNT – 5 mg/m^3, 5h/day, 4 days (C57BL/6)
***Carbon Black – 7 mg/m^3, 6h/day, 5d/week, 13 weeks (B6C3F1)**
#Chrysotile – 10 mg/m^3, 6h/day, 10 days (C57BL/6)

SWCNT – 5 mg/m^3, 5h/day, 4 days (C57BL/6)
^Silica – 43 mg/m^3, 5h/day, 9 days (C3H/HeN)
#Chrysotile – 10 mg/m^3, 6h/day, 30 days (C57BL/6)

*A. Elder et al., 2005. Effect of subchronically inhaled carbon black in three species. 1. Retention kinetiks, lung inflammation, and histology. *Toxicological Sciences* 88 (2):614-629.
^G.S. Davis et al., 1998. Silicosis in mice: Effects of dose, time, and genetic strain. *Journal of Environmental Pathology, Toxicology and Oncology*, 17(2):81-97.
#R.F. Robledo et al., 2000. Increased phosphorylated extracellular signal-regulated.. *Am Journal of Pathology*, 156(4).

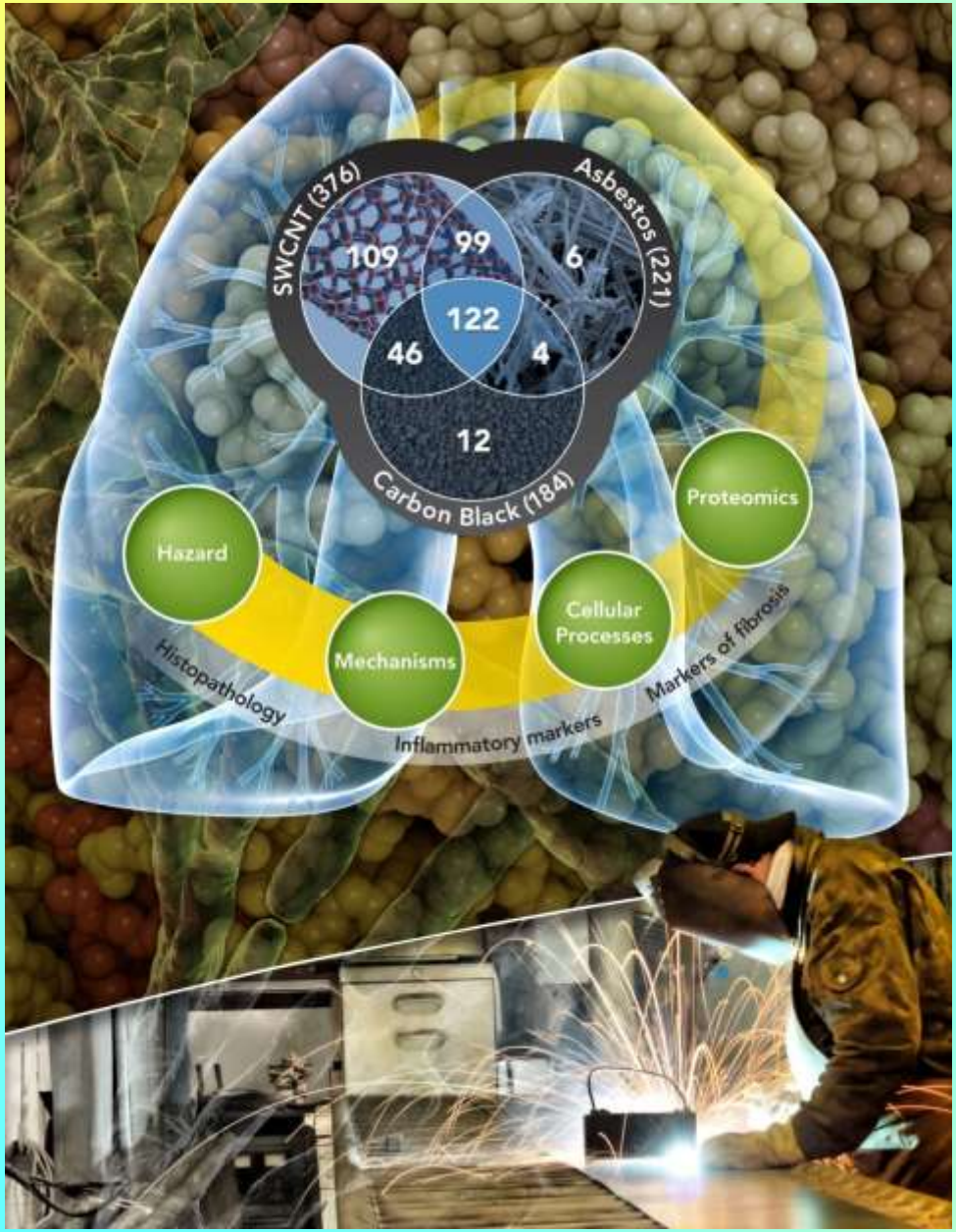
A. Haegens et al., 2007: Minimal fibrosis was detected only after 40 days of inhalation with chrysolite asbestos (7-10 mg/m^3, 6h/day, 40 days), evaluated with Masson's trichrome (C57BL/6).

Pulmonary Responses to SWCNT, Asbestos and UFCB



Conditions: 40 μg/mo, twice a week, 3 weeks, pharyngeal aspiration

Cumulative dose: SWCNT/Asbestos/UFCB = 240 μg/mouse

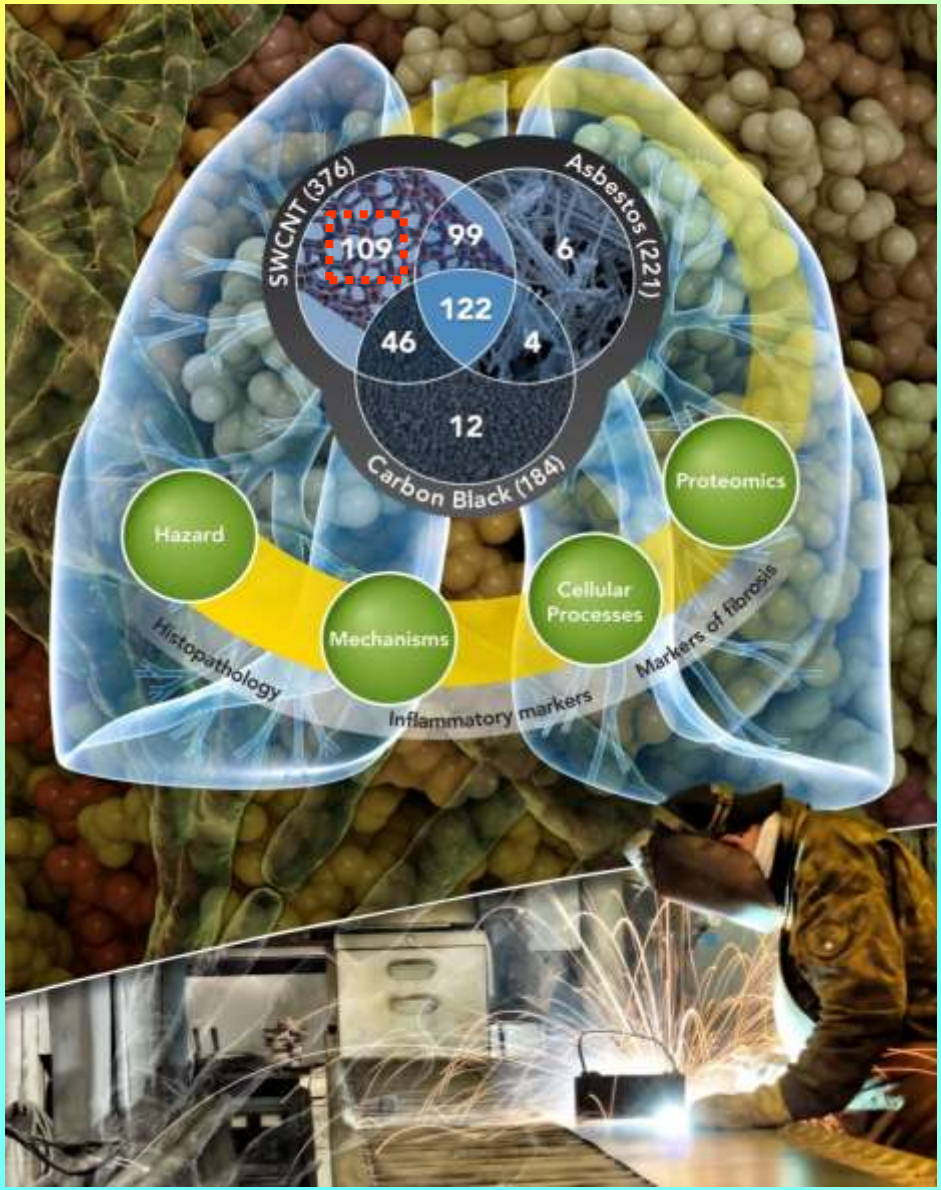


Number of Proteins Common or Unique to SWCNT, Asbestos or Carbon Black Treatment

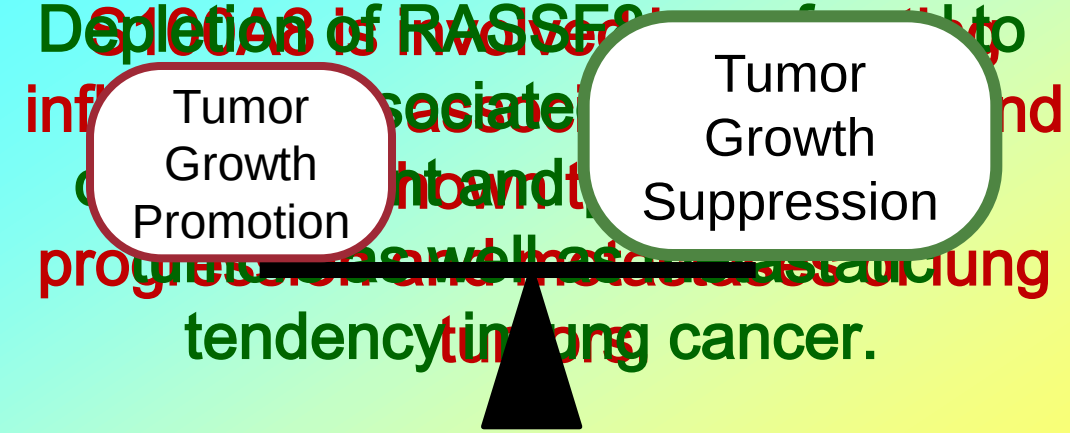
Overall, a significantly smaller number of proteins were affected by Asbestos and Carbon black treatment than for SWCNT

The list of proteins affected by asbestos and carbon black was almost entirely a subset of those affected by SWCNT, implying a strong similarity in the pulmonary response to these materials

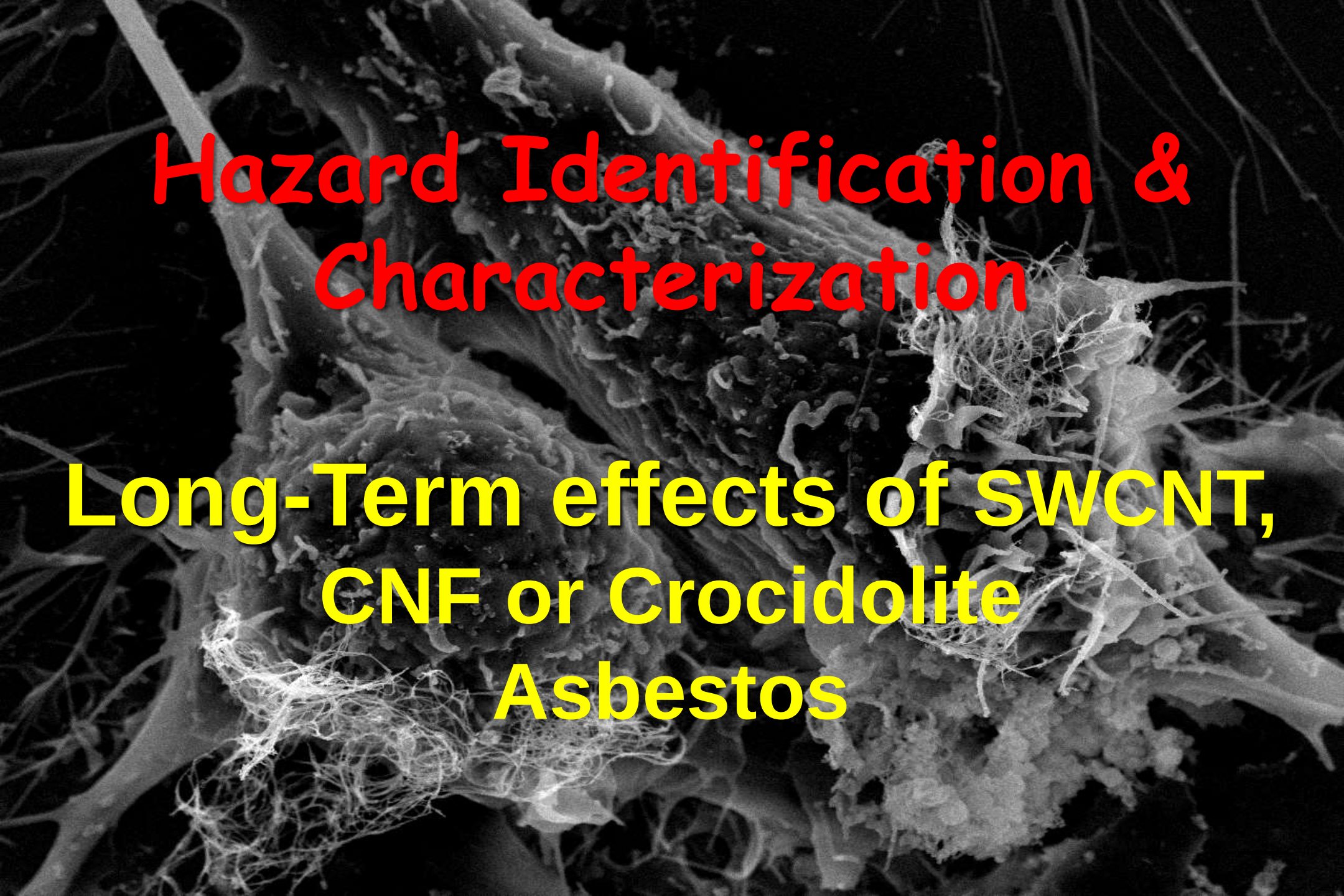
C57BL/6 mice were exposed by pharyngeal aspiration twice a week, for 3 weeks. Cumulative dose - 240 μg/mouse



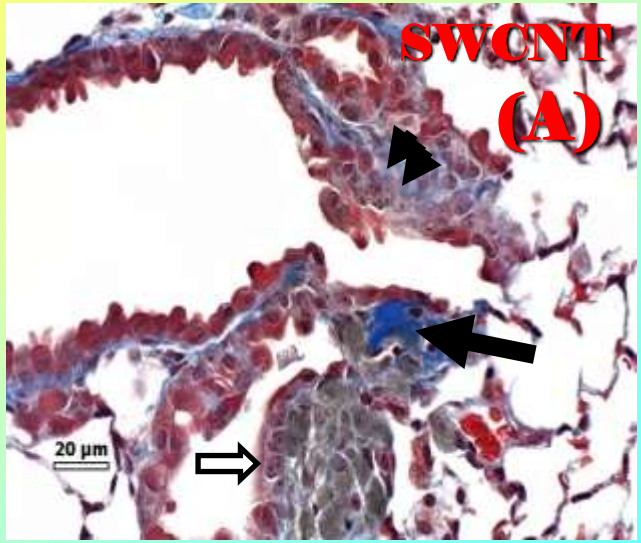
Proteins Unique to SWCNT Exposure (109)	
Involved in Tumor or Metastatic Growth	**Involved in Tumor Suppression**
• Cathepsin-B (CTSB) • Cathepsin-Z (CTSZ) • Integrin Linked Kinase (ILK) • S100 calcium binding protein A8 (S100A8) • Phosphoglycerate Mutase 1 (PGAM1) • Vimentin (Vim)	• RAS-association domain family 8 (RASSF8) • B-cell CLL/lymphoma 6 member B (BCL6B) • Septin7 (SEPT7) • Rho GDP dissociation inhibitor (GDI) beta (ARHGDIB)

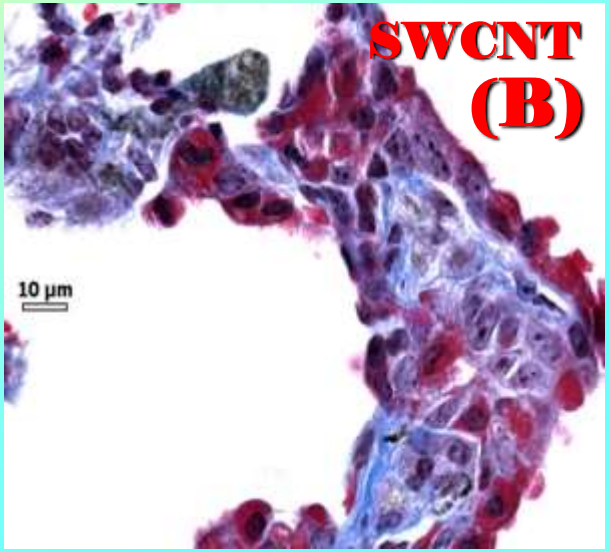


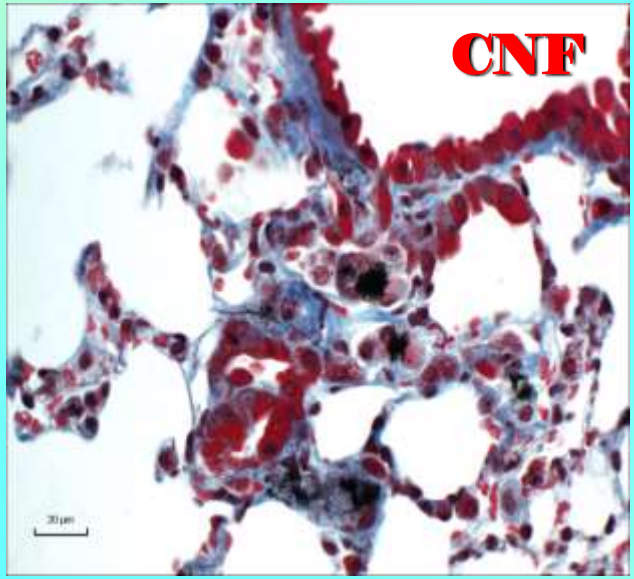
C57BL/6 mice were exposed by pharyngeal aspiration twice a week, for 3 weeks. Cumulative dose - 240 μg/mouse

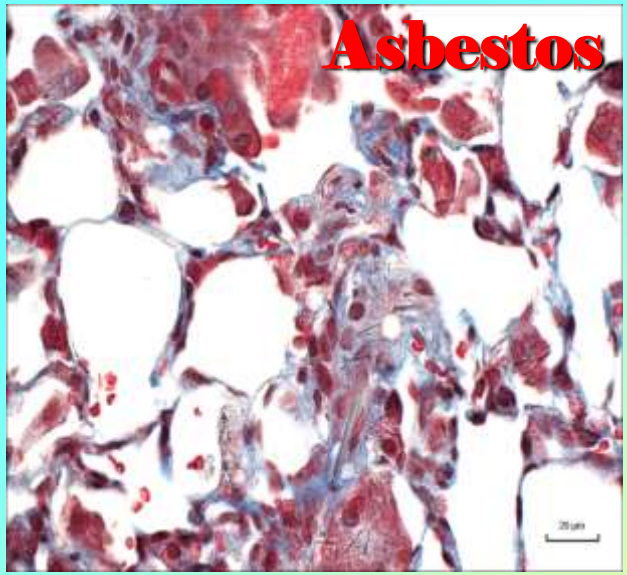

Hazard Identification & Characterization
Long-Term effects of SWCNT, CNF or Crocidolite Asbestos

Chronic Responses Following One Year Post Exposure with SWCNT, CNF or Asbestos









A - persistent granulomatous lesions with abnormal fibrotic response, and significant bronchiolar epithelia cell hypertrophy. Bronchiolarization is apparent as the airway epithelium extended over the SWCNT

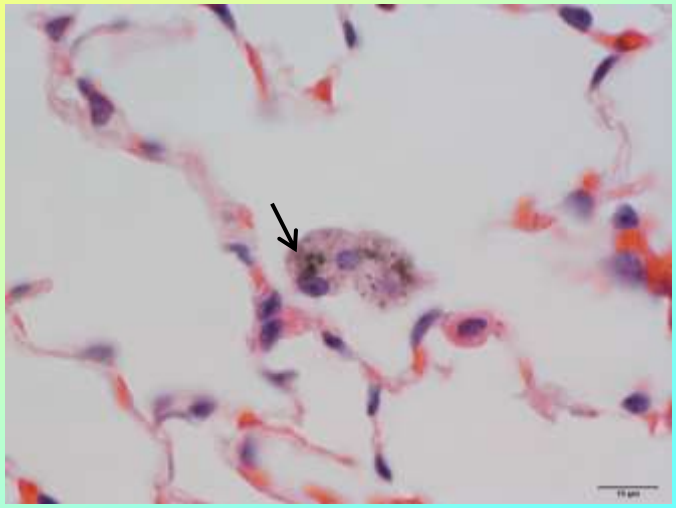
B - granulomatous lesions in the proximal alveolar region of the lungs, the significant hypertrophy, hyperplasia and bronchiolarization due to the chronic persistence of SWCNT in this region of the lungs. SWCNT is infiltrated with collagen and cells

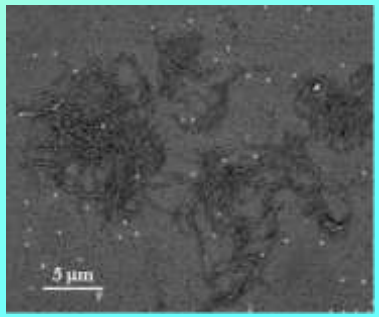
Shvedova et. al., AJPLung, 2013

	SWCNT	CNF	Asbestos
Inflammation	5 of 5	9 of 11	4 of 4
Fibrosis	5 of 5	8 of 11	2 of 4
Osteoid	3 of 5	0 of 11	0 of 4

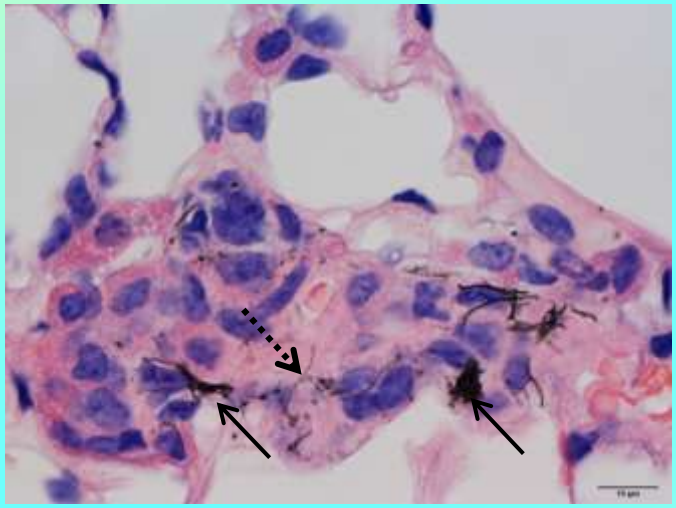
Nanoparticle retention in lungs after one year post exposure

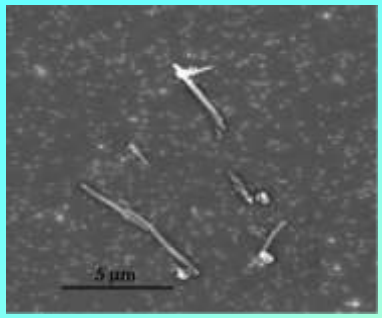
Tangled SWCNTs



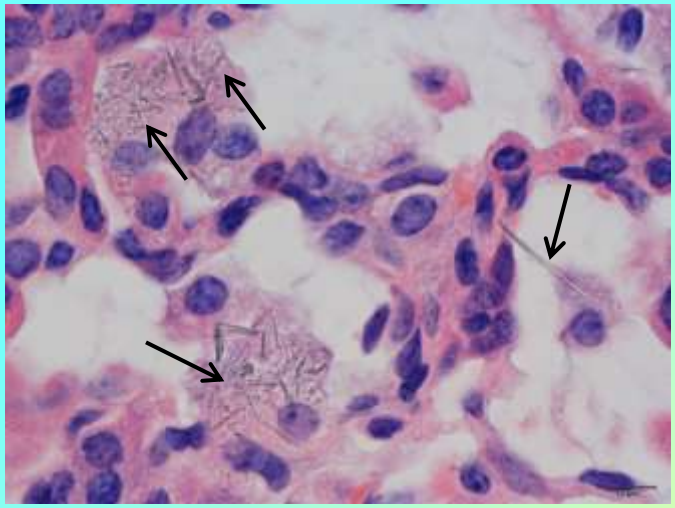


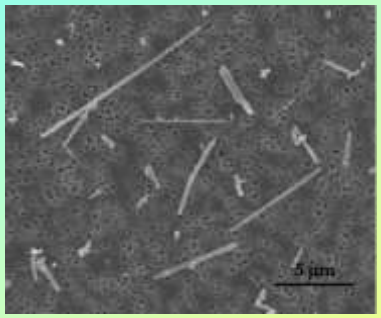
Carbon Nanofibers





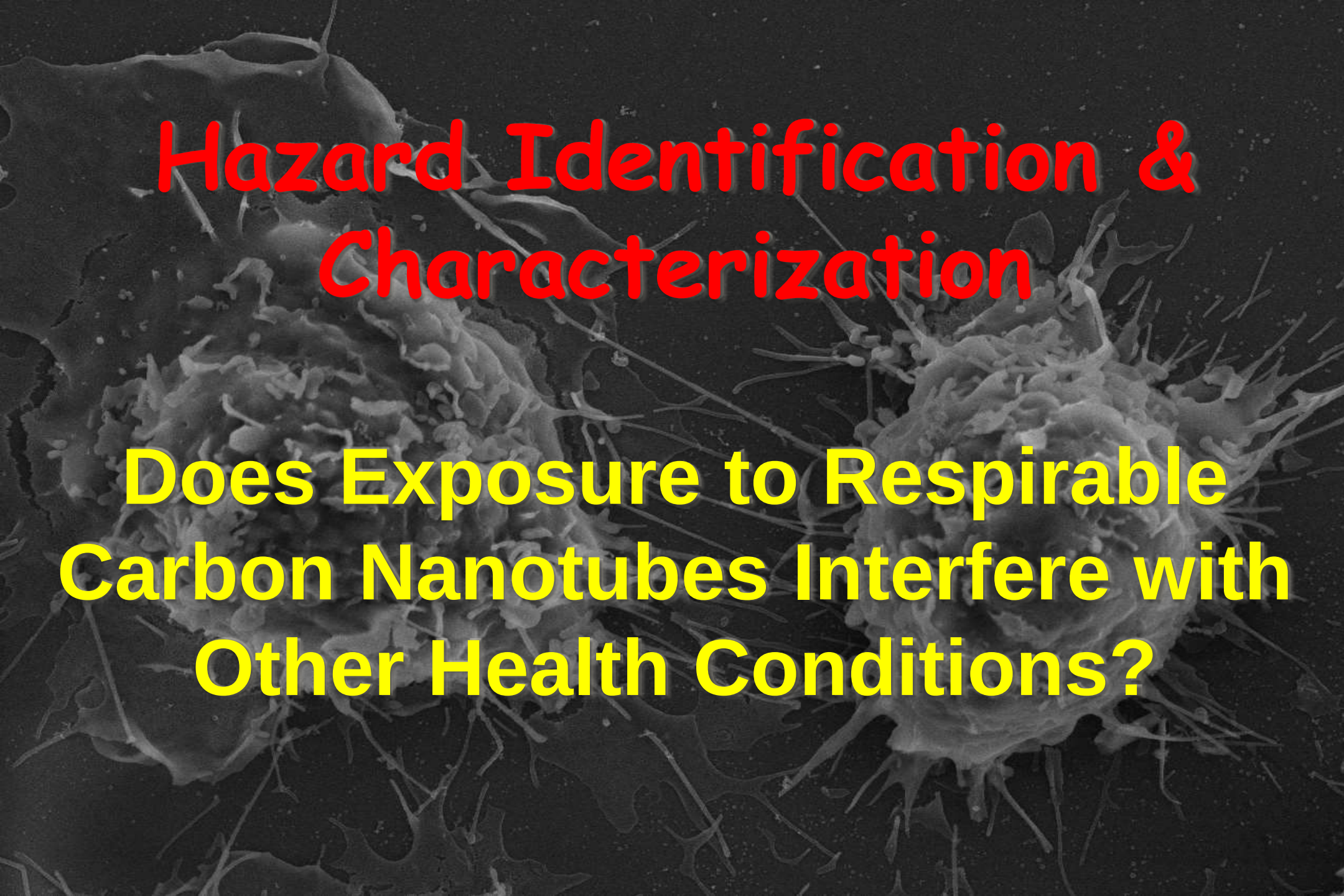
Asbestos





Biopersistance of nanostructures depends on its size, shape, and composition.

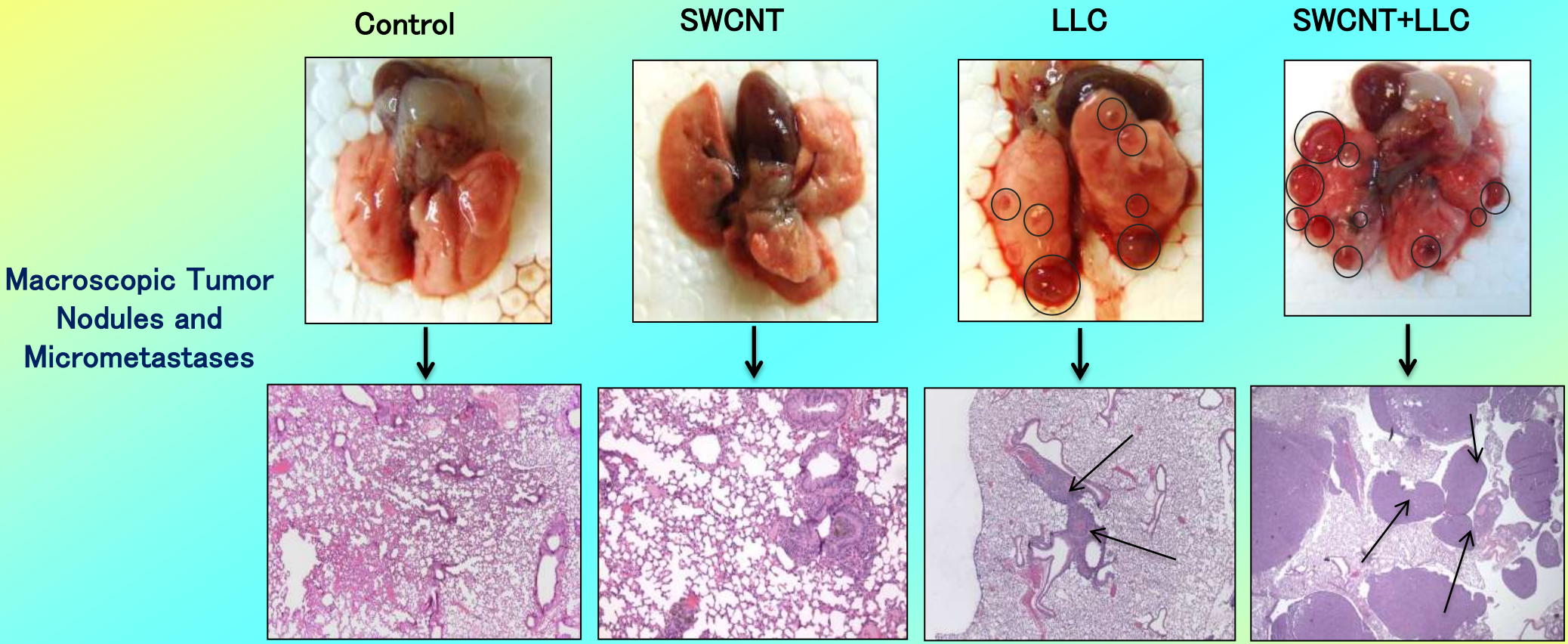
In the case of SWCNTs, Raman microscopy showed that ~5 – 10% of the initial lung burden (40µg) still remained in the lungs after one year post exposure.



Hazard Identification & Characterization

Does Exposure to Respirable Carbon Nanotubes Interfere with Other Health Conditions?

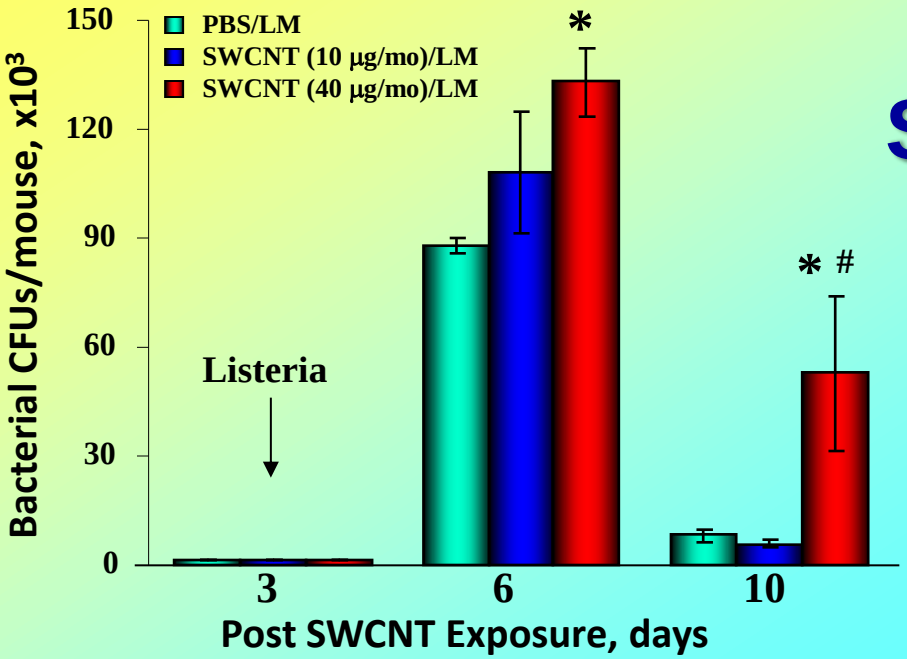
Acute Exposure to SWCNT Accelerated Formation and Growth of Lung Carcinoma

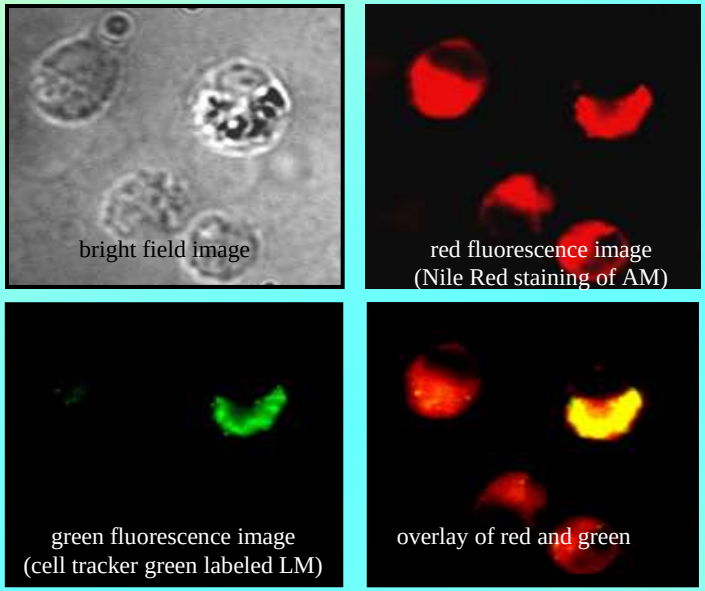


The lung tumors showed characteristic features of poorly-differentiated non-small cell carcinoma, e.g. nuclear pleomorphism and hyperchromasia, high nuclear/cytoplasmic ratio, brisk mitotic activity, extensive apoptosis and small areas of necrosis

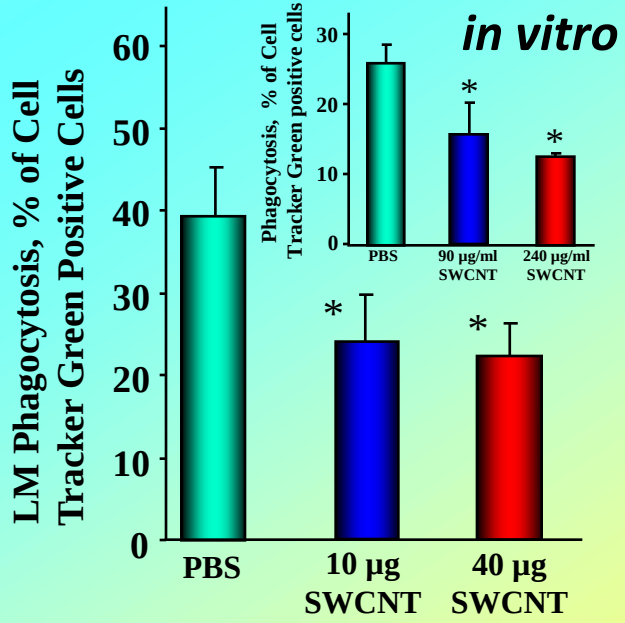
SWCNT did not change the typical morphological features of tumor, but accelerated tumor growth and the appearance of intratumoral necrosis zones, associated with rapid tumor growth

Exposure to Respirable SWCNT Reduced Pulmonary Bacterial Clearance in Lung of C57BL/6 Mice





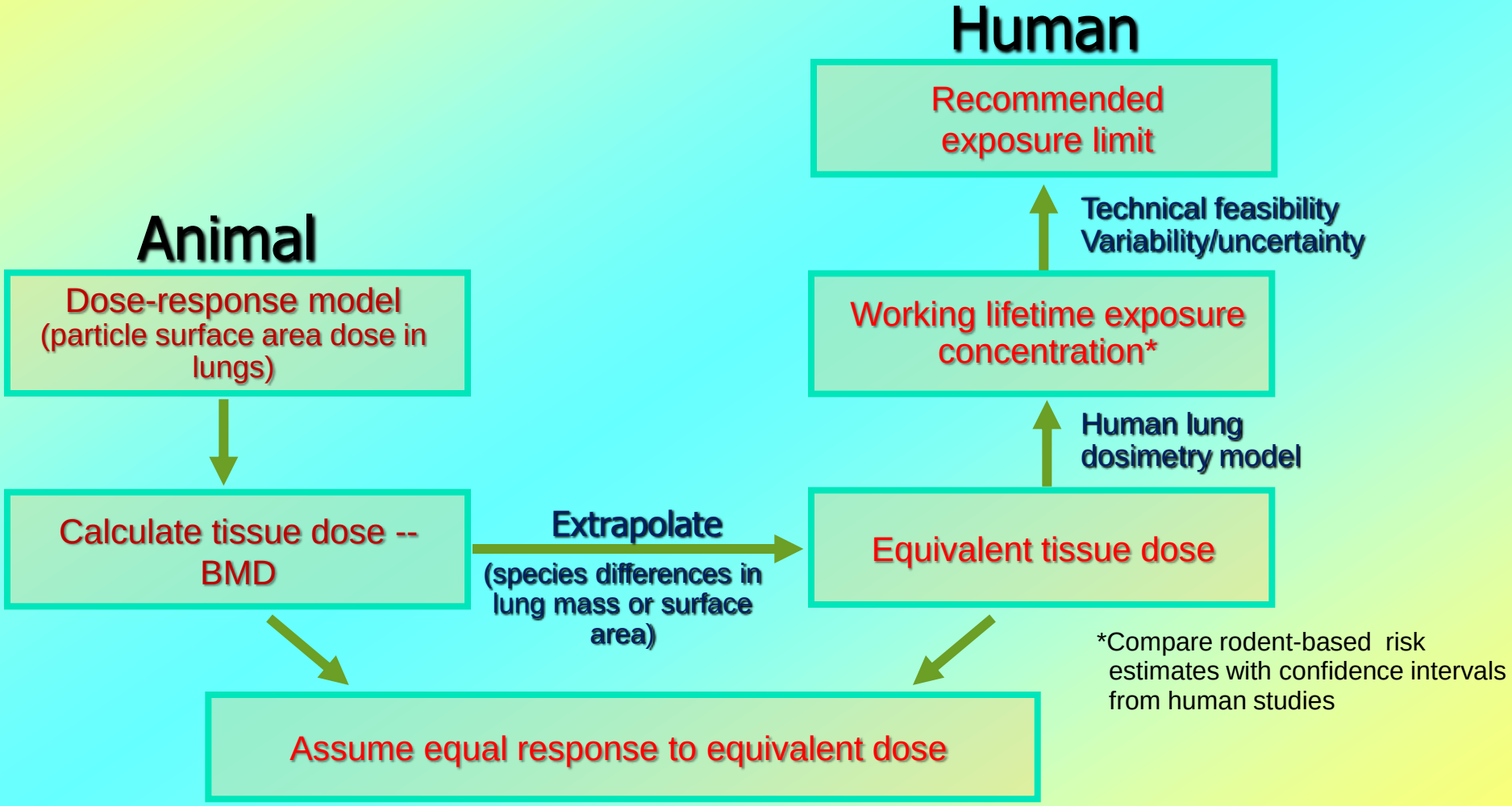
Typical Fluorescence Micrograph of AM Pre-Treated with SWCNT and Co-Incubated with LM



Exposure to SWCNT Altered Ability of Alveolar Macrophages to Phagocytose Listeria monocytogenes

Risk Assessment

Quantitative Risk Assessment in Developing Recommended Exposure Limits for Nanomaterials



Based on Kuempel et al. [2006]

Risk Analysis using Available Pulmonary Toxicology Data

A. Calculate lung burden in rodent models
B. Normalize lung burden/alveolar epithelial surface area
    1. Human = 102 m^2
    2. Rat = 0.4 m^2
    3. Mouse = 0.05 m^2

(Stone et al. Am J Respir Cell Mol Biol. 6: 235-243, 1992)

Calculate Equivalent Lung Burden and Airborne Nanoparticle Levels

Example Scenario : MWCNT

Lung Burden = Air Level x Ventilation[a] x Duration x Deposition Fraction[b]

(μg) = ($\mu g/m^3$) x (m^3/d) x (5d/w x 50w/y x 45 yr) x 10%

[a] Ventilation / 8 hr working = 9.6 m^3/d (ICRP, 1994)

[b] Deposition fraction for MWCNT having CMAD ~420 nm = 10% (Task Group Lung Dynamics. Health Phys 12: 173-190, 1966)

Calculation of Benchmark Workplace Level for Human Exposure

Rodent endpoints granulomatous inflammation or fibrosis with SWCNT or MWCNT (10% risk)

Study	Exposure	CNT	Benchmark Exposure Level ($\mu g/m^3$)
Lam et al., 2004	Intratracheal (mice)	SWCNT	10.0
Shvedova et al., 2005	Aspiration (mice)	SWCNT	1.80
Shvedova et al., 2008	Inhalation (4d) (mice)	SWCNT	0.11
Muller et al., 2005	Intratracheal (rat)	MWCNT	18.0
Porter et al., 2010	Aspiration (mice)	MWCNT	0.61
Ma-Hock et al., 2009	Inhalation (13w) (rat)	MWCNT	0.50

Carbon Nanotube: Exposure Limit Recommended by NIOSH

"While there are no scientific reports of 'adverse health effects in workers producing/using carbon nanotubes… or carbon nanofibers'", NIOSH also expressed concern over the potential or worker exposure.

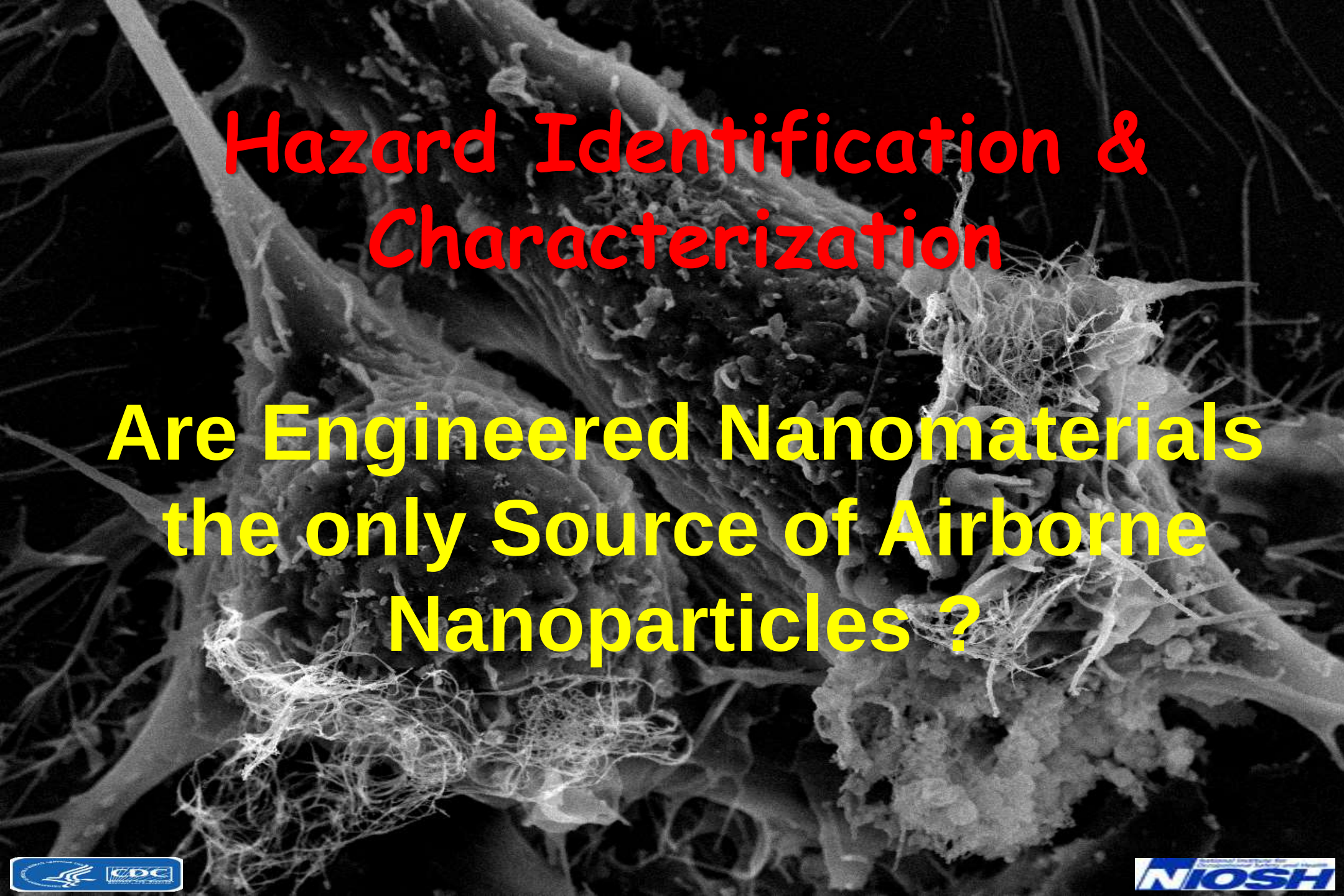
NIOSH Recommends exposure limit for CNT (REL) is set at **1 µg/m³**

NIOSH explained the benchmark by indicating the "REL is based on the available sub-chronic and short-term animal dose-response data of early-stage fibrotic and inflammatory lung response to CNT exposure

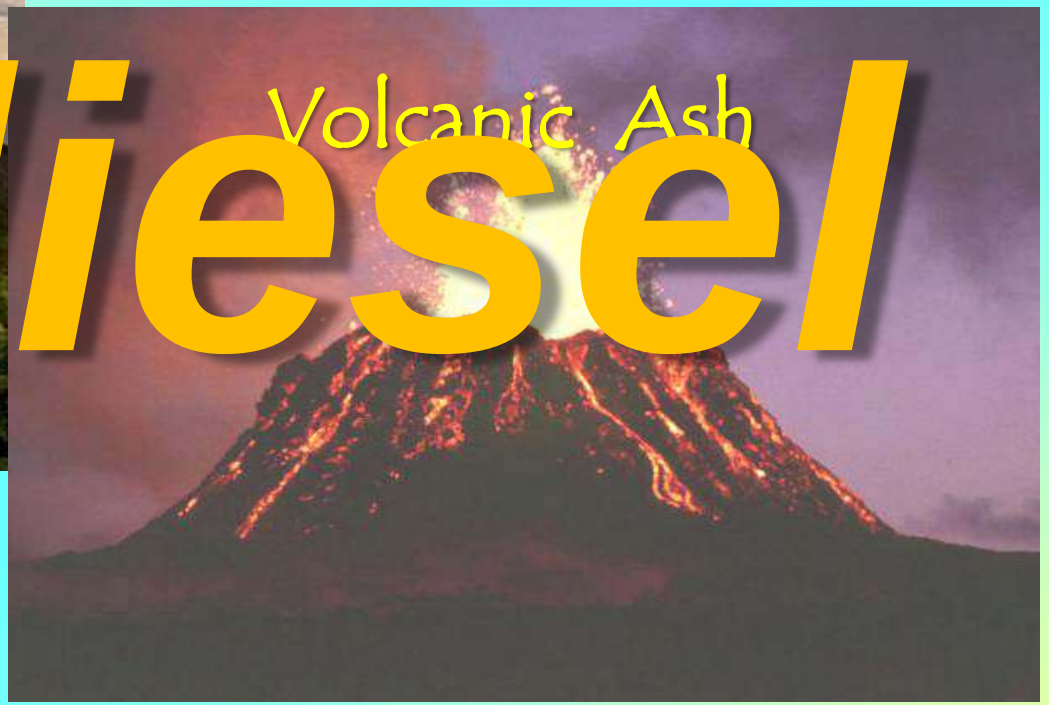
Benchmark dose (BMD) estimates from the animal data (95% lower confidence limit estimates of the BMD) have been extrapolated to humans by accounting for species differences in alveolar lung surface area.

Working lifetime exposure concentration have been calculated based on estimates of either the deposited or retained alveolar lung dose of CNT assuming an 8-hour time-weighted average (TWA) work shift exposure during a 40-hour work week, 50 weeks /year, for 45 years

http://www.cdc.gov/niosh/docket/review/docket161A/default.html


Hazard Identification & Characterization
Are Engineered Nanomaterials the only Source of Airborne Nanoparticles ?

Nanosized Materials Found in Nature



Biodiesel

Nanosized Materials are also Formed during Combustion Processes

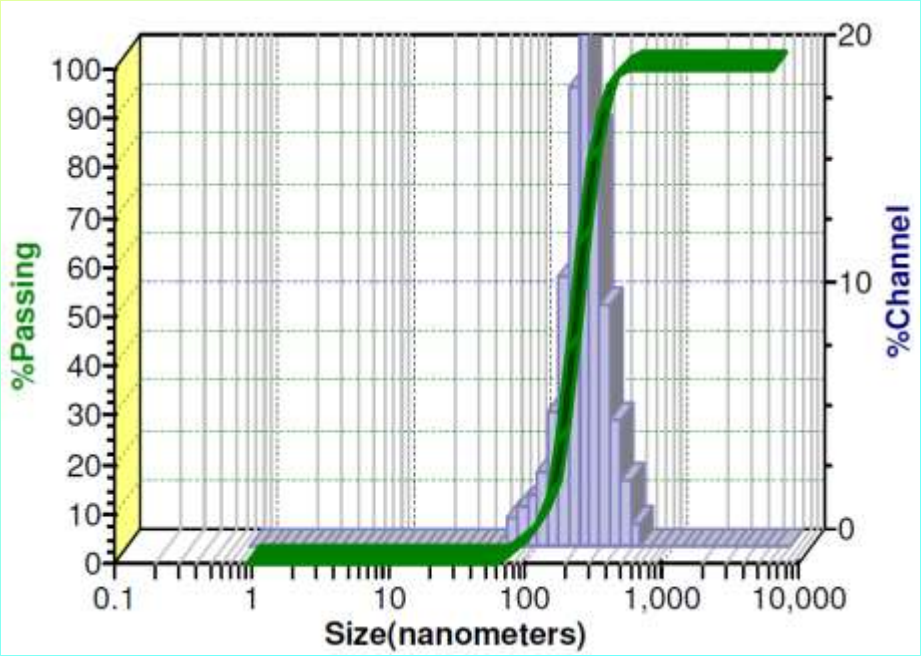




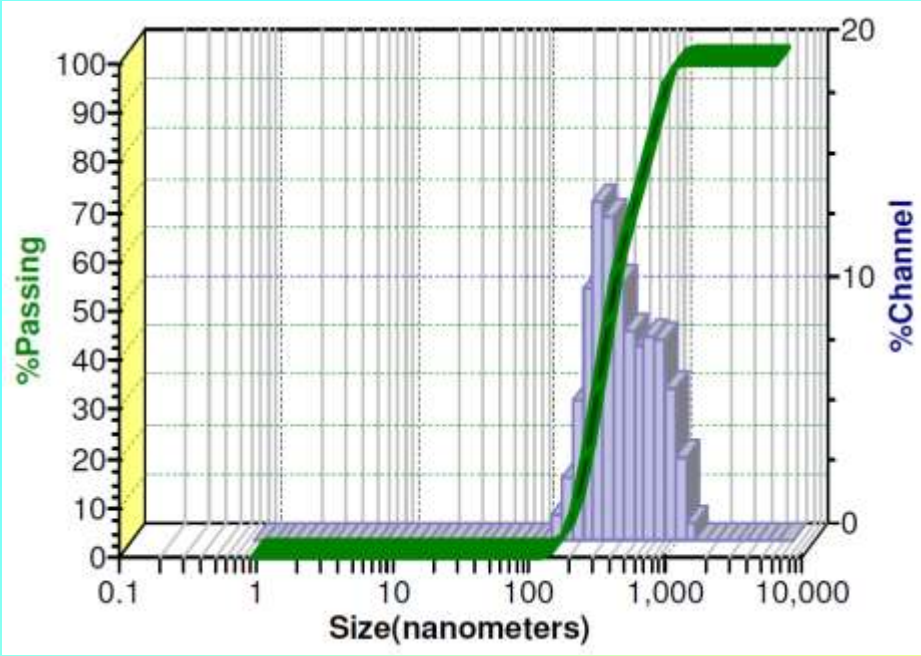



Size Distribution Profiles of Exhaust Particles
(Dynamic Light Scattering)

Biodiesel (B)



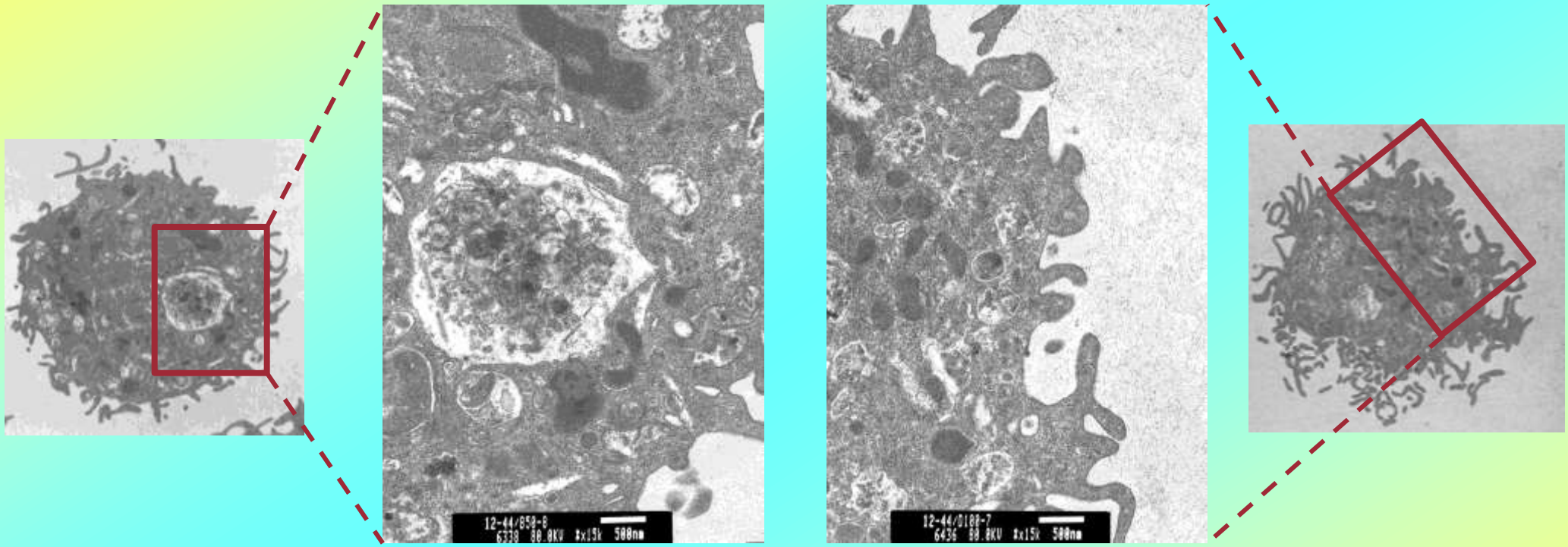
Diesel (D)



The size of exhaust particles from biodiesel aerosol collection ranges between 80 - 500nm and diesel between 200nm – 1.4μm.

TEM of BALF MΦs 7 days post exposure

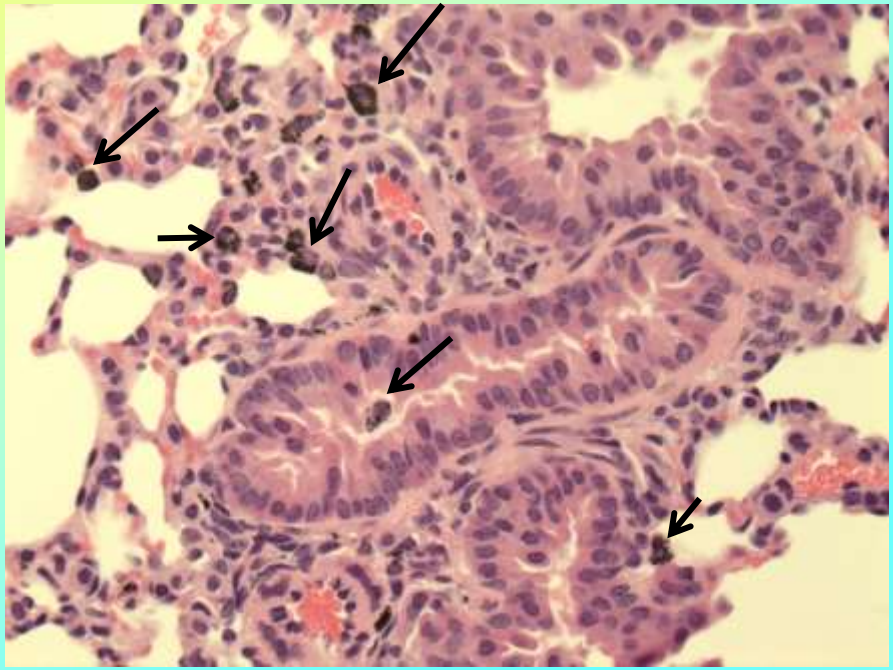
Biodiesel (B) **Diesel (D)**



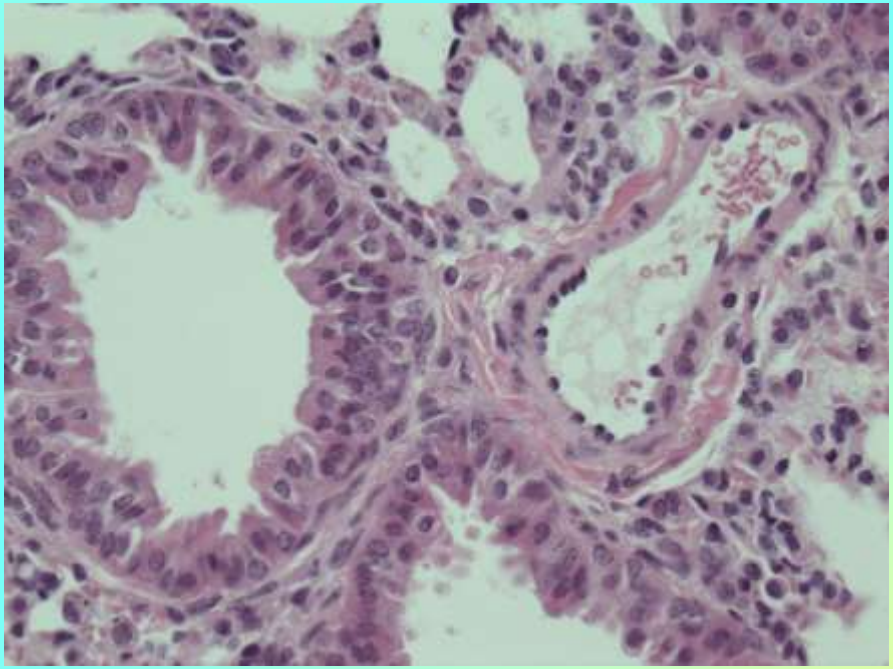
Electron micrographs indicate localization of biodiesel PM to spherical organelles (ranging 1-2μm in diameter) in MΦs, mimicking lipid droplets.

Lung histology 28 days post exposure

Biodiesel (B)

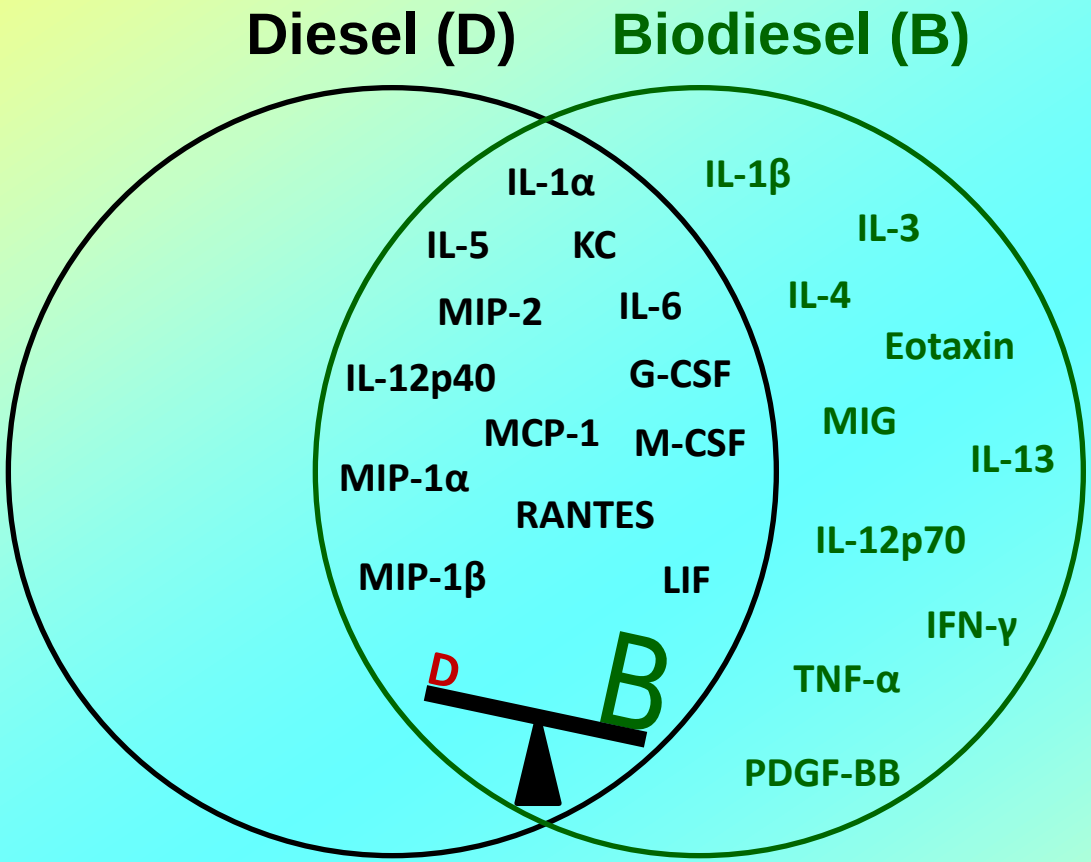


Diesel (D)

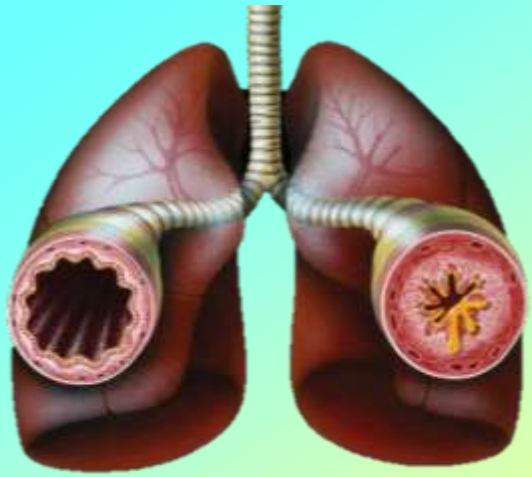


Lung sections indicated presence of lymphocytic infiltrate and impaired clearance with prolonged retention of biodiesel PM in pigment laden macrophages (as indicated by arrows). No significant inflammation was observed after 28 days post exposure to D exhaust.

Inflammatory Mediators: Differential Responses



Asthma



Accumulation of cytokines - IL-1β, IL-4, IL-13, IL-12p70, IFN-γ, TNF-α - seen only upon B exposure could be associated with allergic inflammation and induction of type 1 (Th1) and type 2 T helper cell (Th2) responses.

What is Lacking?

1. **Relevance of acute and chronic effects observed in the lungs in relation to other organ systems and tissues (systemic effects).**
2. **Characterization of nanomaterial toxicity on the basis of physicochemical properties as well as its mode of action.**
3. **Relevance of *in vitro* and *in vivo* screening tests to worker response to inhalation of ENMs.**
4. **Predictive algorithms for structure/function relationships and comparative toxicity analyses for risk assessment.**
5. **Human biomarkers of nanomaterial exposure and/or responses.**

How can NIOSH contribute to such studies?

Predictive models for risk assessments.

1. **Evaluate adverse effects using model systems**
   - **Short & long term exposure studies using rodents**
   - **Co-culture Systems**
   - **In vitro and Ex vivo models that can mimic: (a) Organ System Environments & (b) Real exposure scenarios.**
   - **Engineered tissue models**

2. **Systematically investigate nanoparticles of different physical and chemical properties for their toxicity.**

3. **Develop computational methods that can identify key chemical and physical factors that contribute to toxicity**

4. **Develop predictive algorithms for structure/function relationships and comparative toxicity analyses for risk assessment**
   - Feature Selection & Data Mining
   - Classification algorithms

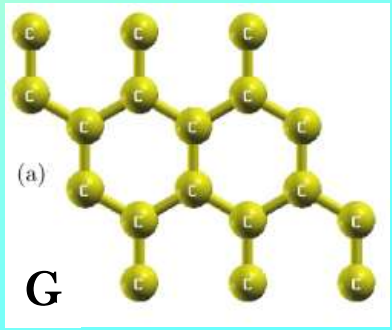
Predictive models for risk assessments

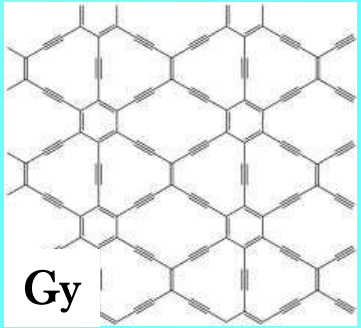
System Biology approaches to identify biomarkers of nanomaterial exposure

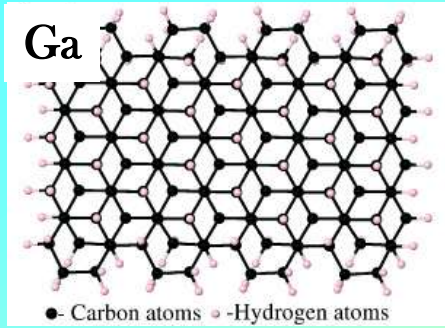
1. Microarray Analysis
2. Epigenetic Studies
3. Proteomics
4. Lipidomics
5. Computational models – interactions of nano-objects with biomolecules/structures

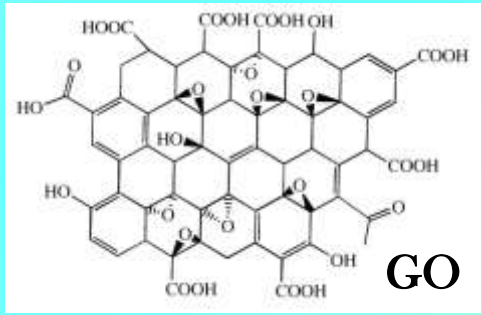
OTHER NANOMATERIALS INVESTIGATED & FUTURE FOCUS

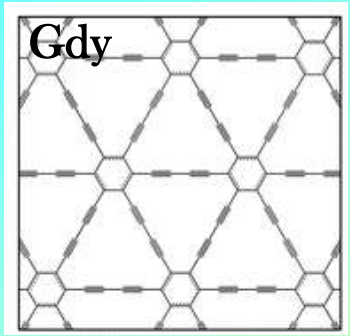
- Biodiesel & Diesel based Nanoparticles
- Graphene (G)
  - Graphene oxide (GO)
  - Graphane (Ga)
  - Graphyne (Gy)
  - Graphdiyne (Gdy)
- Silicene
- Nanocellulose

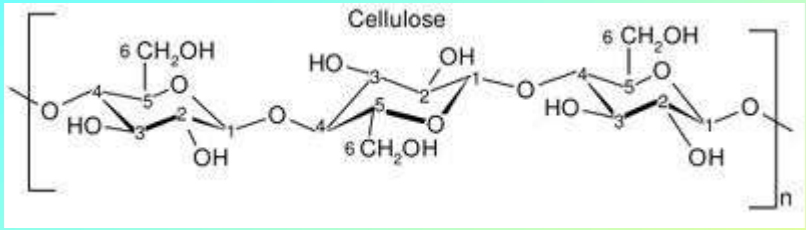


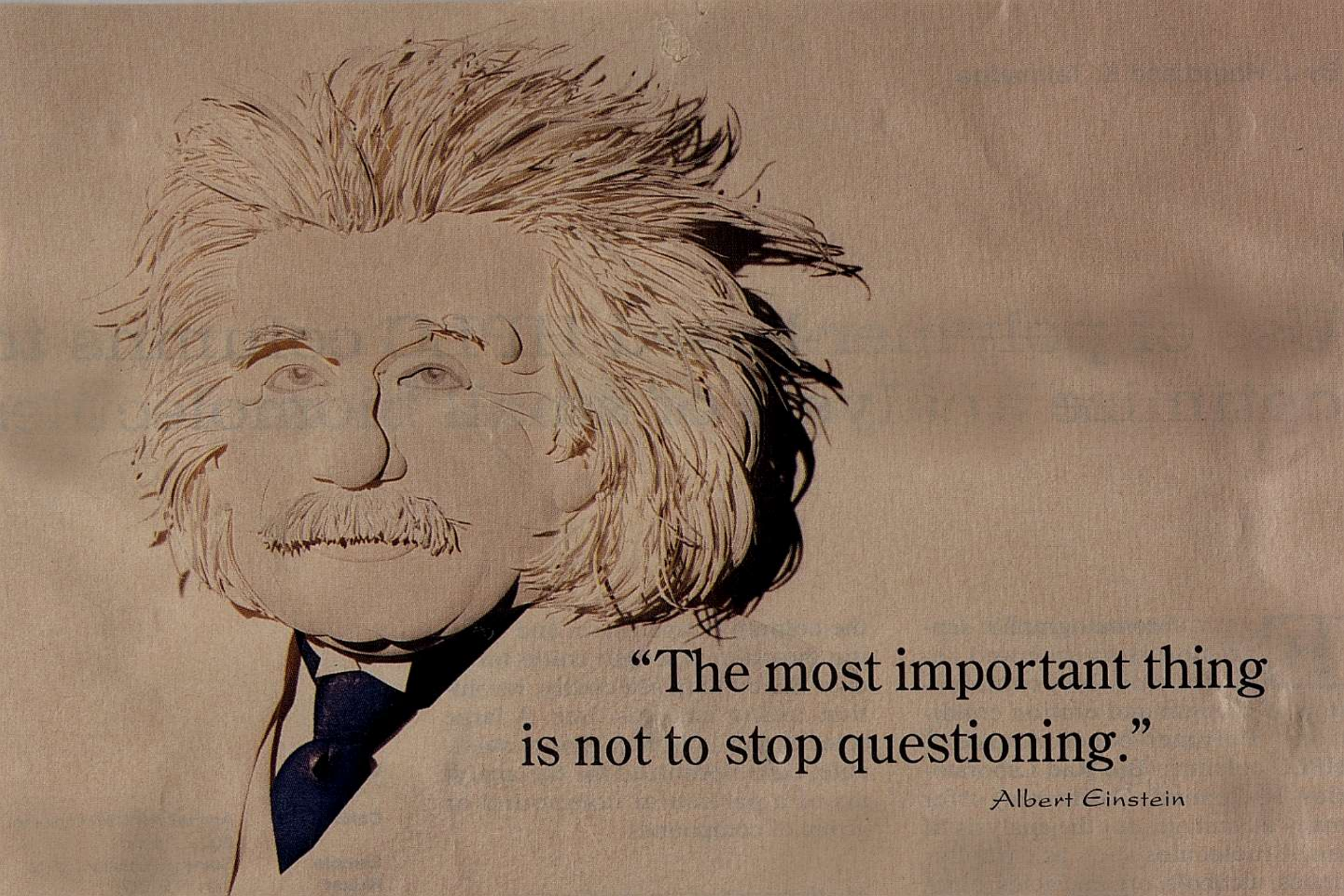











"The most important thing
is not to stop questioning."
Albert Einstein

Thanks To My Collaborators:

N. Yanamala
E. Kisin
A. Murray
M. Farcas
V. Castranova
R. Mercer
P. Baron
G. Deye
B. Chen
A. Hubbs
A. Maynard

V. Gandelsman
O. Gorelik
S. Arepalli

I. Gilmour
S. Gavett

V. Kagan
V. Tyurin
W-H Feng
Y. Tyurina
K. Phoutong
N. Konduru
H. Bayir
A. Star

B. Fadeel
A. Scheynius
E. Witasp
H. Vallhov

M. Kadiiska
R. Mason

Acknowledgments: NIOSH OH008282, NIH HL70755, NORA 927000Y,
7th Frame work program (NANOSOLUTIONS, #309329)

NSF, Arlington, VA
December 2-3, 2013