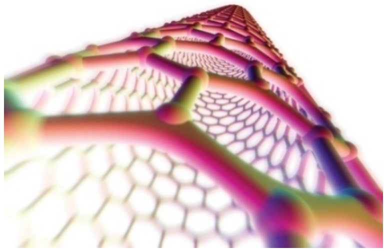
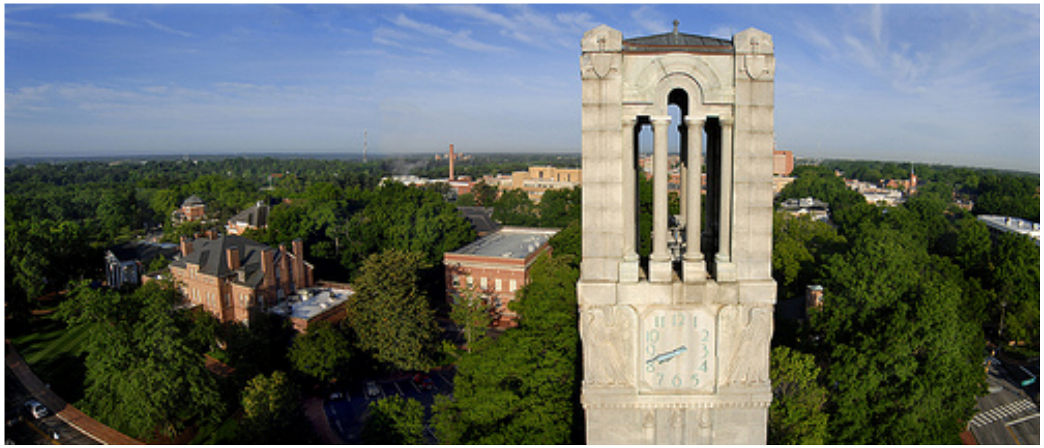
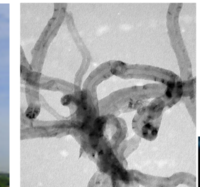
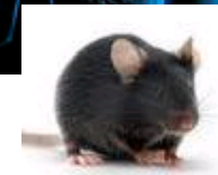
NC STATE UNIVERSITY

# Mechanisms of Susceptibility to Carbon Nanotube Lung Disease









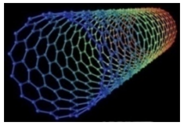

James Bonner, PhD

Toxicology Program, Department of Biological Sciences

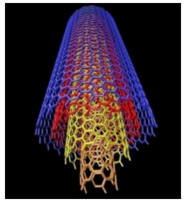
North Carolina State University


20th ETH Conference on Combustion Generated Nanoparticles, June 13-16th, 2016, ETH Zentrum, Zürich, Switzerland

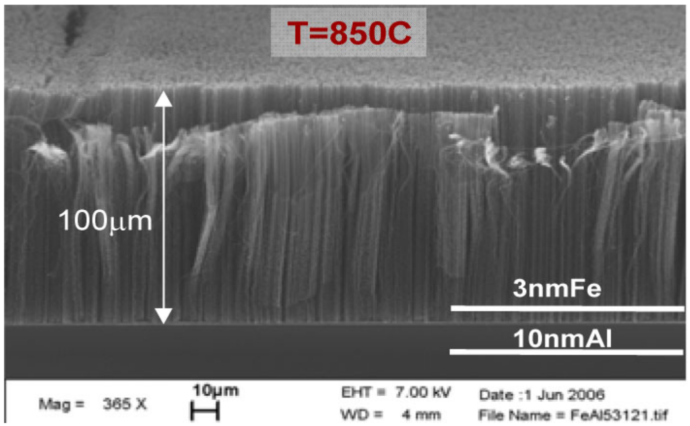
# Carbon Nanotubes

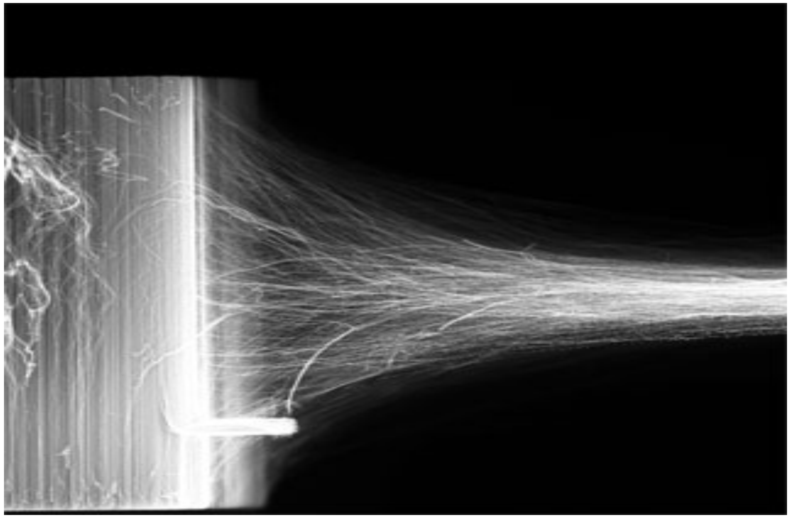


SWCNT



MWCNT

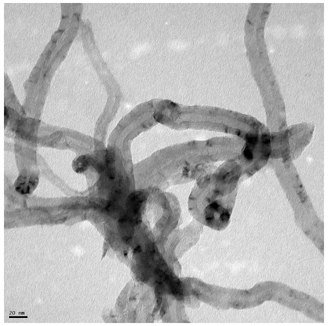


## Synthesis:

•Chemical Vapor Deposition
•Metal Catalysts (e.g., Fe, Ni, Co)
•Numerous Surface Modifications (e.g., carboxylation, atomic layer coatings with metal oxides)

## Applications:



•Nanoscale electronics
•Energy cells
•Drug delivery
•Imaging
•Materials (light-weight super-strong)
•Tissue engineering

# Outline of Topics Covered

## Engineering of Carbon Nanotubes

- Synthesis, purification, and functionalization
- Diversity of types

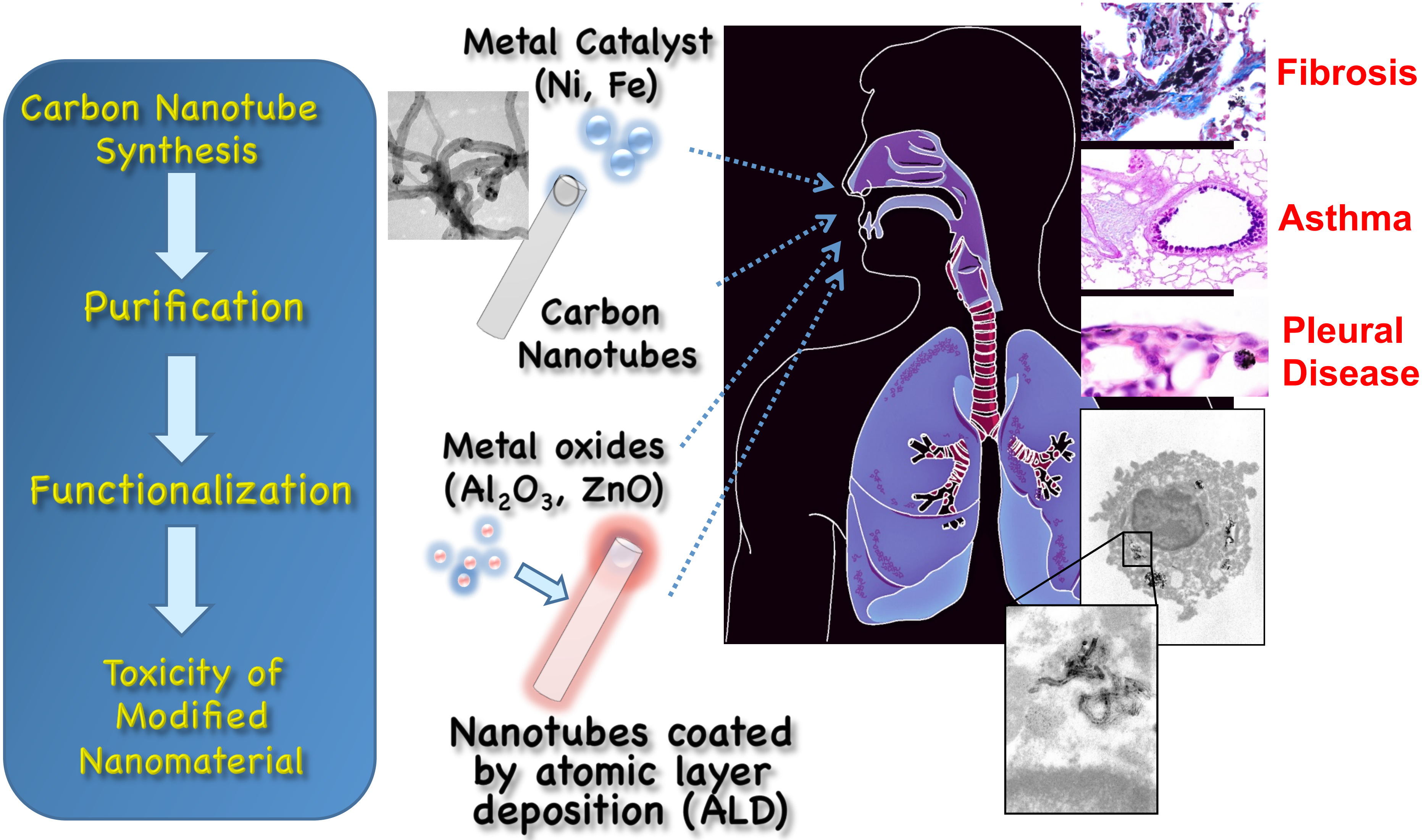
## Exposures to Carbon Nanotubes

- Human: occupational, consumer, anthropogenic
- Mouse: inhalation, aspiration, intranasal

## Potential for human disease

- Pulmonary Fibrosis, mesothelioma, asthma

# Exposure Risks for Lung Diseases During Synthesis and Functionalization of Carbon Nanotubes (CNTs)

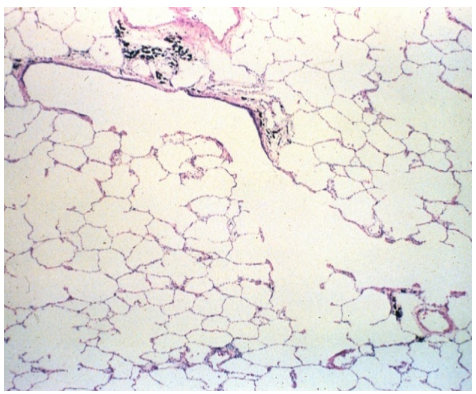


# Defining the Relative Risk of Carbon Nanotubes

No or low risk

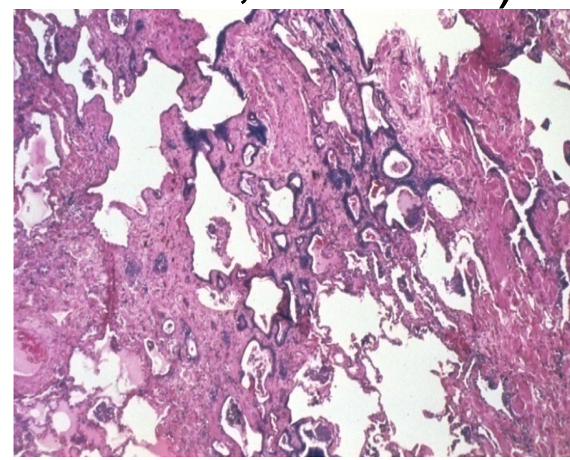
Nanotubes as a single type?

Defined risk (e.g. asbestos: Cancer, Fibrosis)



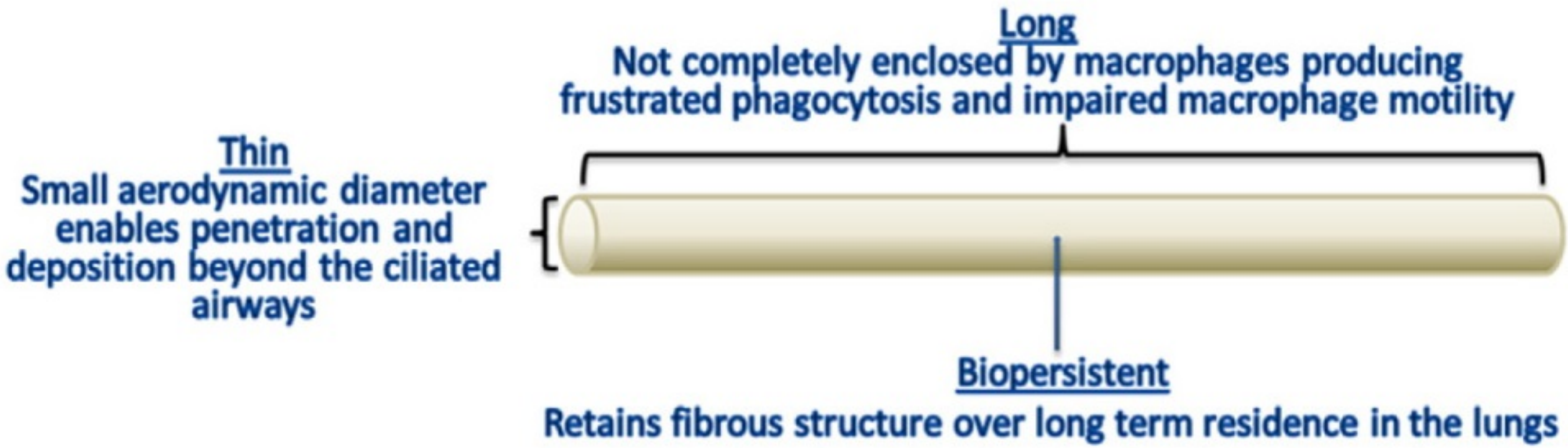
Healthy Lung

Nanotubes as a diverse class?



Asbestosis Lung

# Similarities Between Carbon Nanotubes and Asbestos

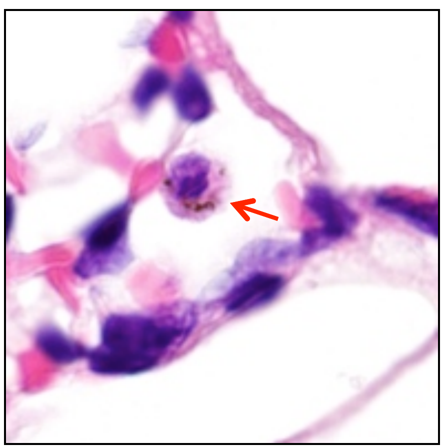



*"In terms of pathogenicity and mechanism CNTs produce oxidative stress, inflammation, genotoxicity, and fibrosis. These are similar to asbestos "*

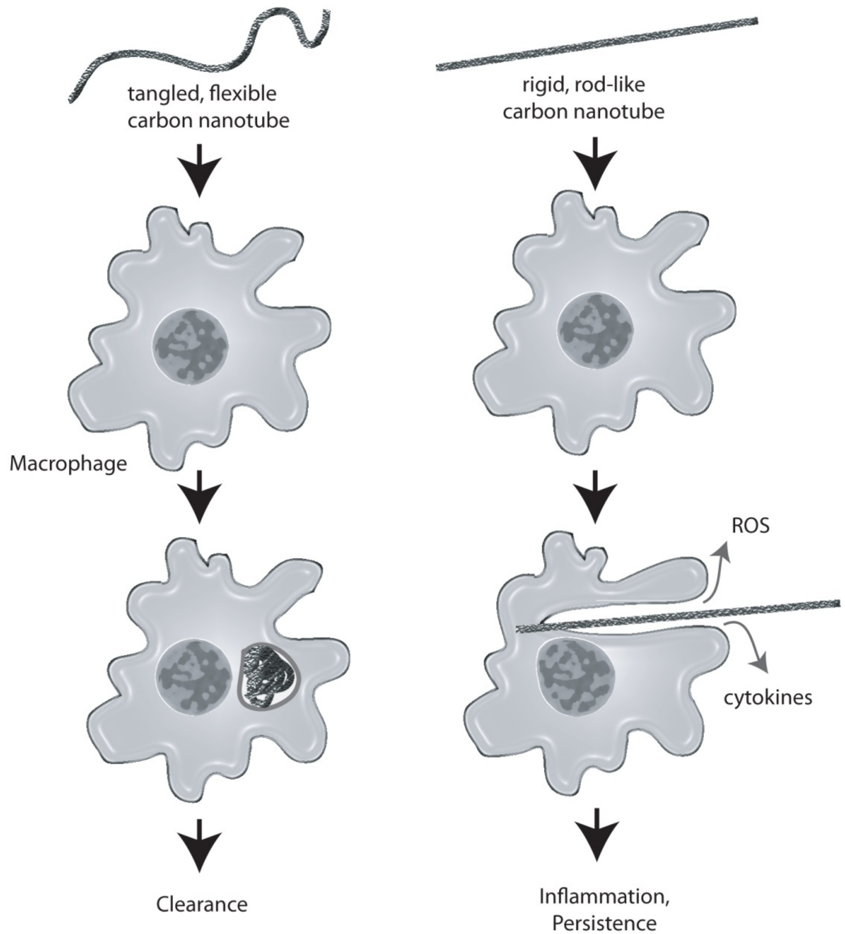
*"The effects of CNTs as particles (i.e., short or tangled CNTs) would be limited to the lungs (fibrosis & cancer), whereas CNTs as fibers would affect lung and pleura (fibrosis & mesothelioma)"*

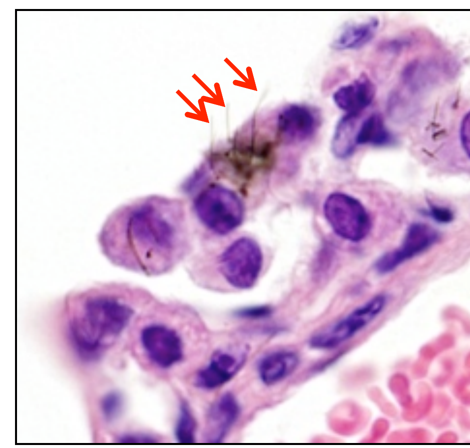
Donaldson et al., 2013. Pulmonary toxicity of carbon nanotubes and asbestos: Similarities and differences. Adv. Drug Delivery Rev. 65, 2078-2086.

# Physico-Chemical Characteristics that Predict Carbon Nanotube Toxicity



Helix MWCNT

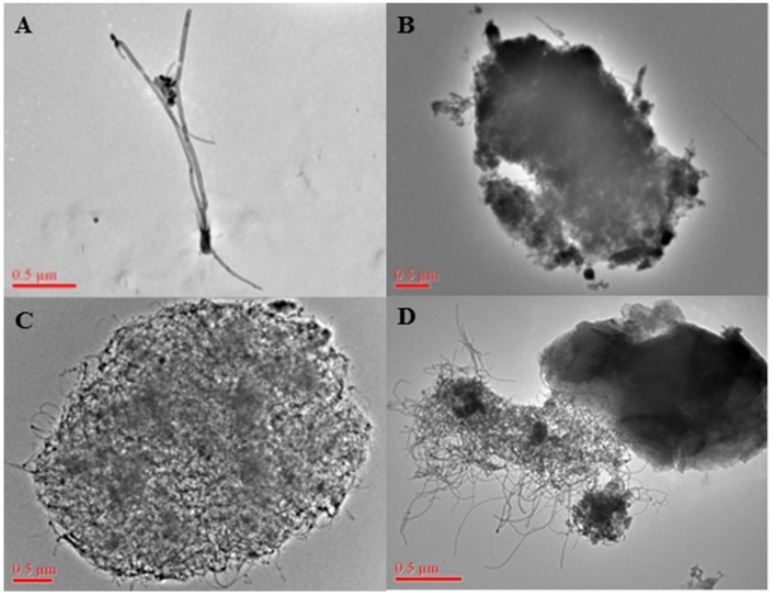


Mitsui-7 MWCNT

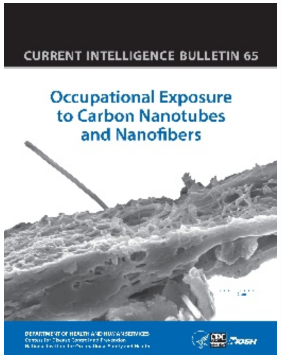
*Bonner JC. Nanotoxicology. In Molecular & Biochemical Toxicology, 5th Ed. In press.*

# Workplace Exposure to Carbon Nanotubes




*Eight MWCNT manufacturing facilities evaluated; exposures ranged from non-detectable samples to 80 µg/m$^3$*

NIOSH recommended exposure limit (REL) of 1 µg/m$^3$ 8-hour time-weighted average (TWA) concentration




*"These findings showed a limited pulmonary inflammatory potential of MWCNT at levels corresponding to the average inhalable elemental carbon concentrations observed in U.S.-based CNT facilities and estimates suggest considerable years of exposure are necessary for significant pathology to occur at that level."*

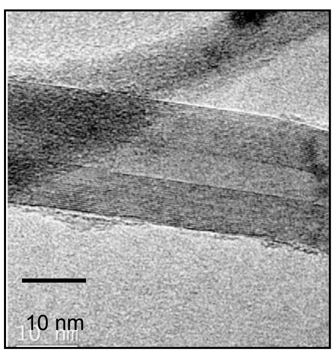
*Erdely et al., 2013 Carbon nanotube dosimetry: from workplace exposure to inhalation toxicology. Part. Fibre Tox. 10:53.*

NC STATE UNIVERSITY

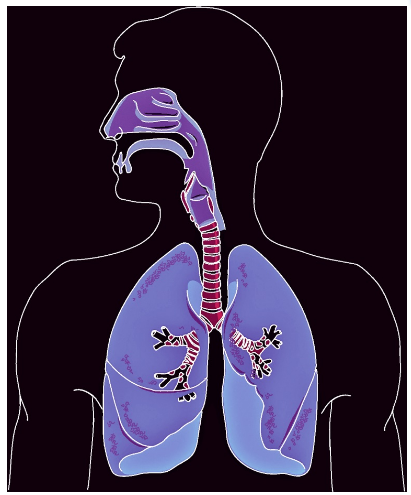
# Relative Human Health Effects: Engineered Carbon Nanotubes (CNTs) vs Combustion-Derived CNTs

## Engineered

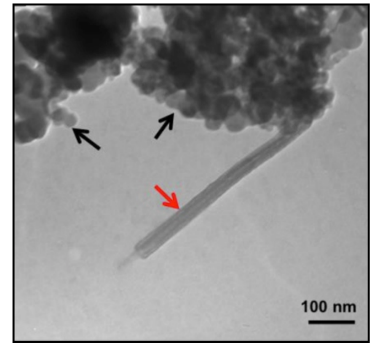

*Ryman-Rasmussen et al. Nature Nanotech. 2009, 4:747-1751.*



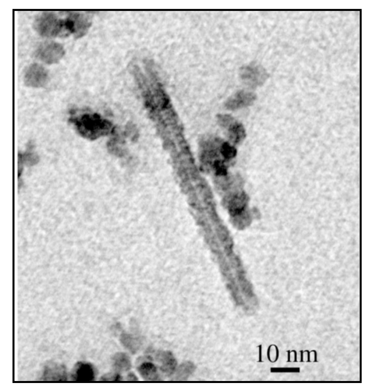

*Porter et al. Toxicology 2010,269:136-147.*



## Combustion-Derived

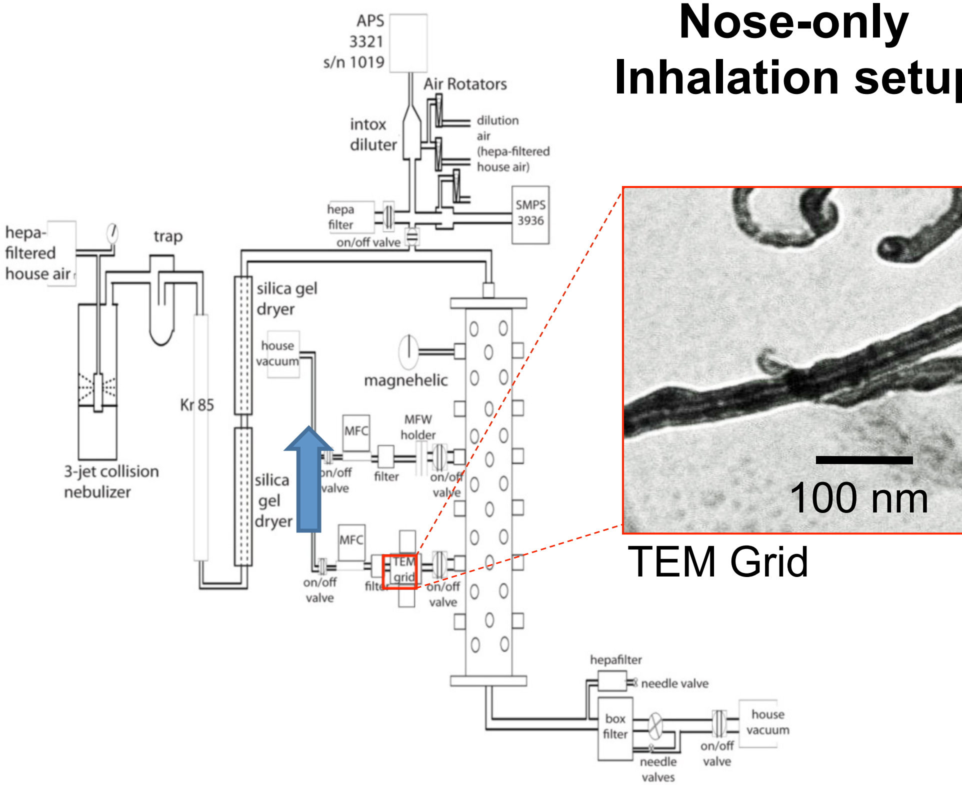



*Kolosnjaj-Tabi et al. EBioMedicine 2015, 2:1697-1704.*



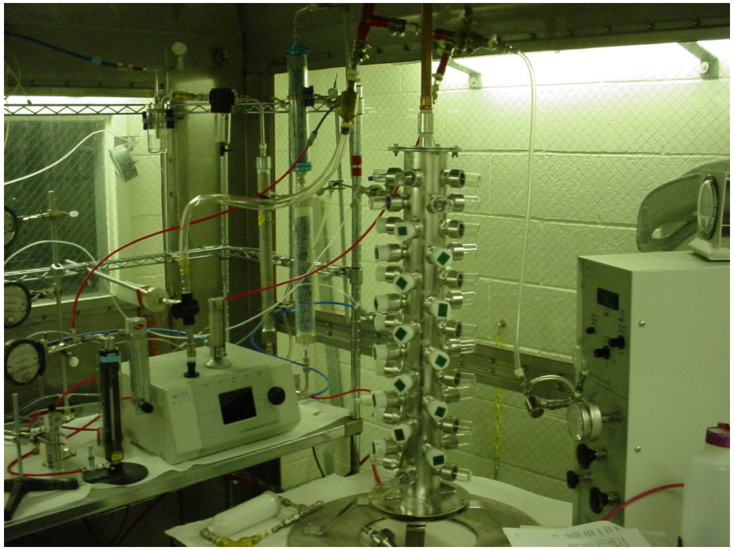

*Jung et al. et al. J. Air Waste Manag Assoc. 2013, 63: 1199-1204.*

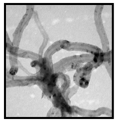
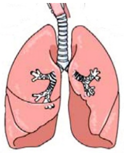
# Inhalation of Carbon Nanotubes in Mice

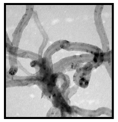
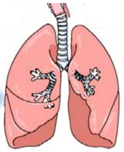



**Nose-only Inhalation setup**

TEM Grid

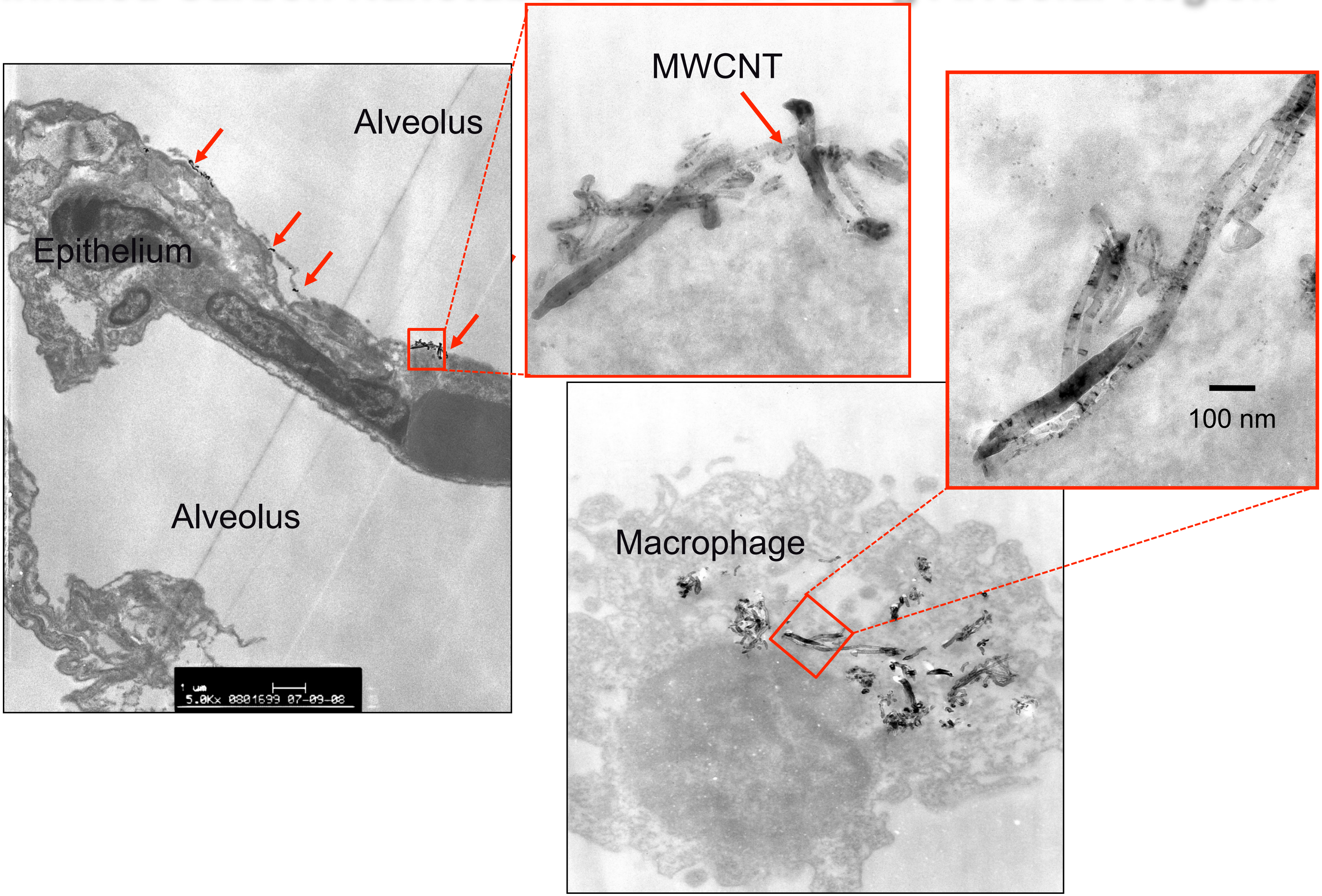


**High-dose, nose-only**  →  **6 hr, 1 day, (1 or 30 mg/m$^3$)** →  **1 wk – 6 mth**

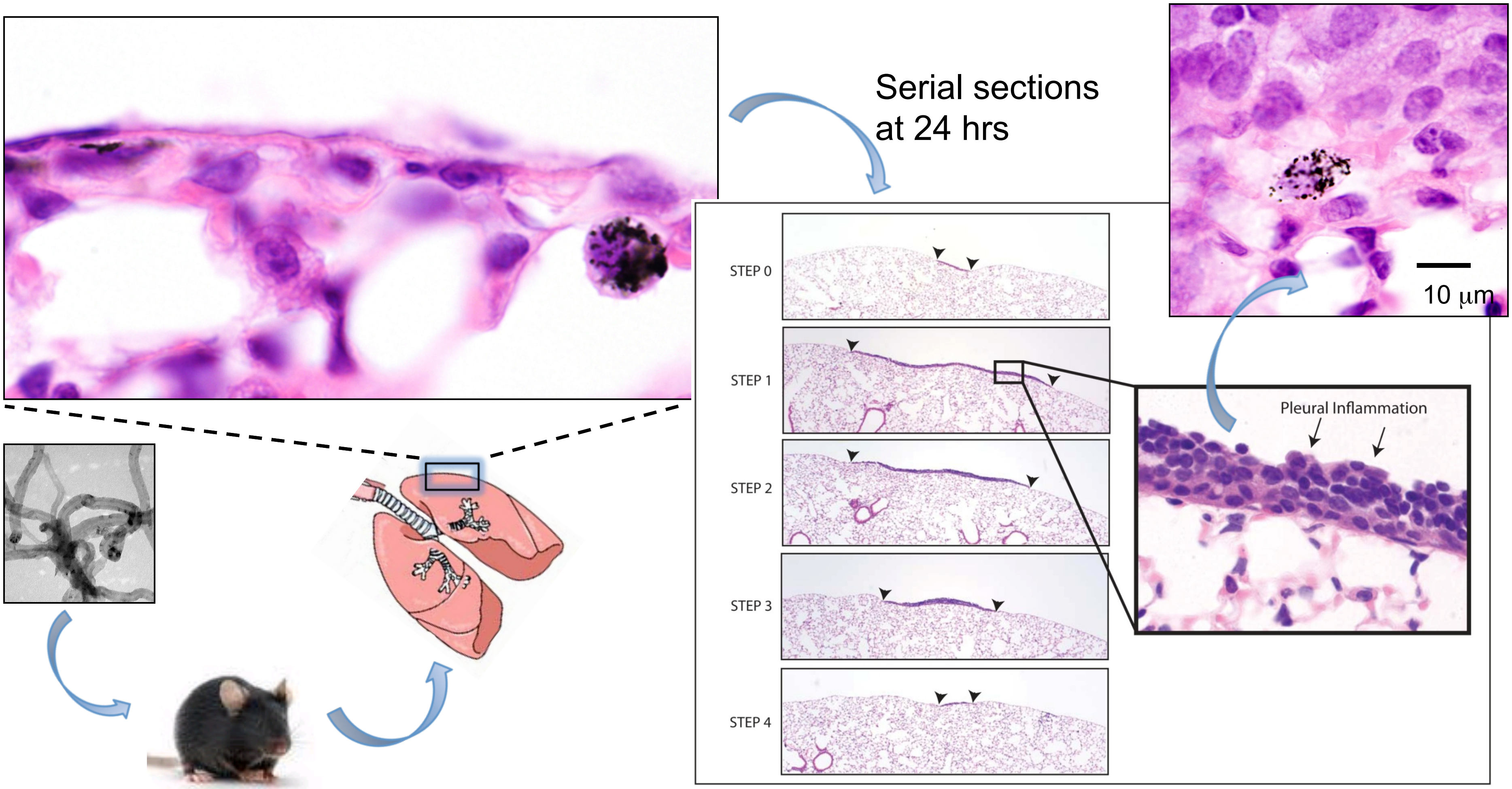
**low-dose, whole body**  →  **6 hr, 5 day/wk, 30 days (0.06, 1, or 0.6 mg/m$^3$)** →  **3 – 30 dy**

*Ryman-Rasmussen et al., Nature Nanotech. 2009 Nov;4(11):747-51.*

# Inhaled Carbon Nanotubes in Mouse Lung Alveolar Region

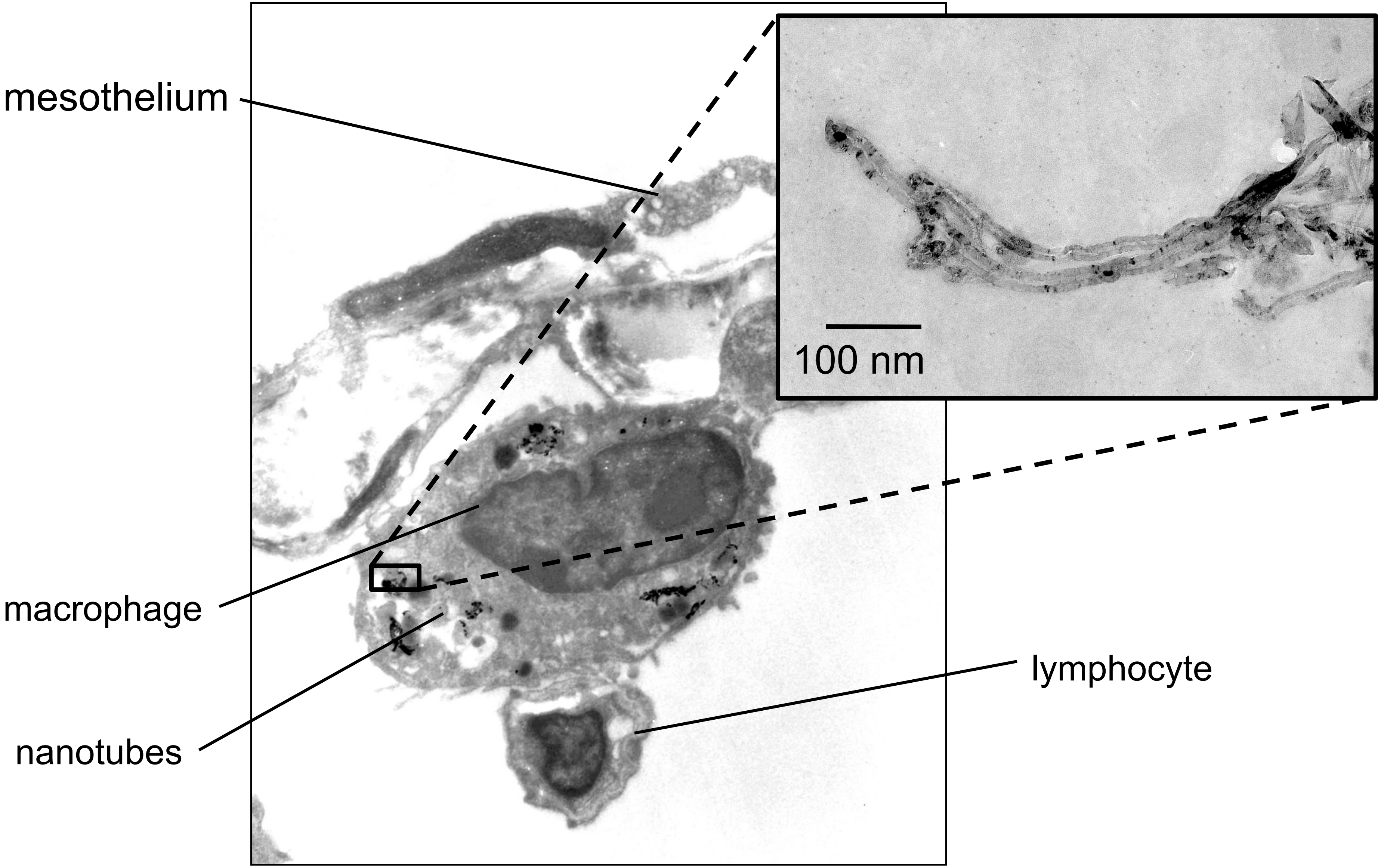


# Acute Pleural Inflammation in Mice after MWCNT Inhalation




*Ryman-Rasmussen et al., Nature Nanotech. 2009 Nov;4(11):747-51.*

# MWCNT Persistence at Pleura after 3 Months




Ryman-Rasmussen et al., Nature Nanotech. 2009 Nov;4(11):747-51.

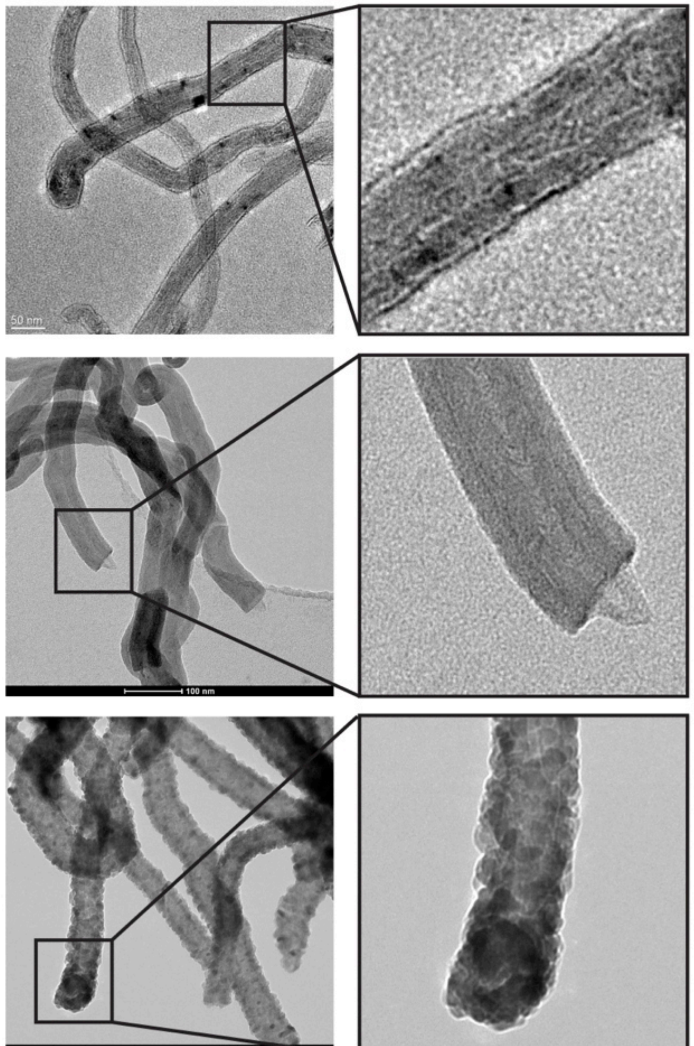
NC STATE UNIVERSITY

# Functionalization of Carbon Nanotubes to Enhance Specific Physico-Chemical Properties in Engineering

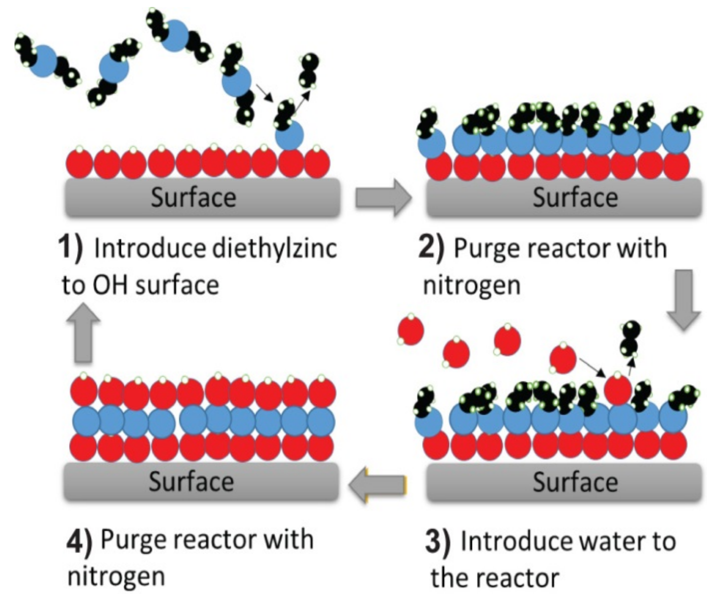
Uncoated MWCNT (Helix, Inc.)

$Al_2O_3$-Coated (A-MWCNT)

ZnO-coated (Z-MWCNT)



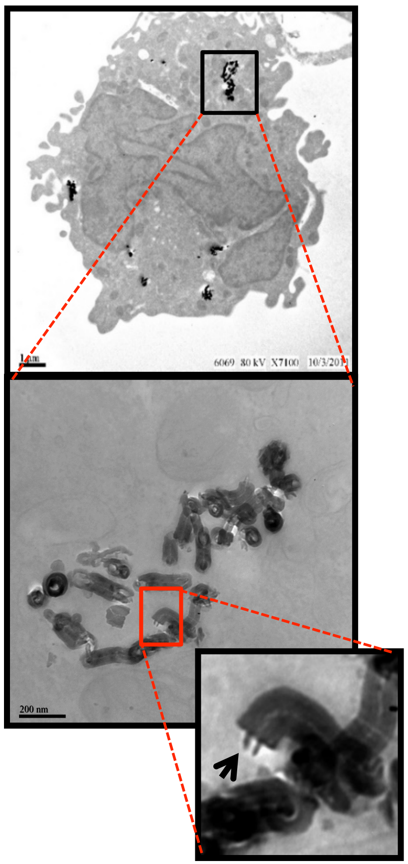
## Atomic Layer Deposition



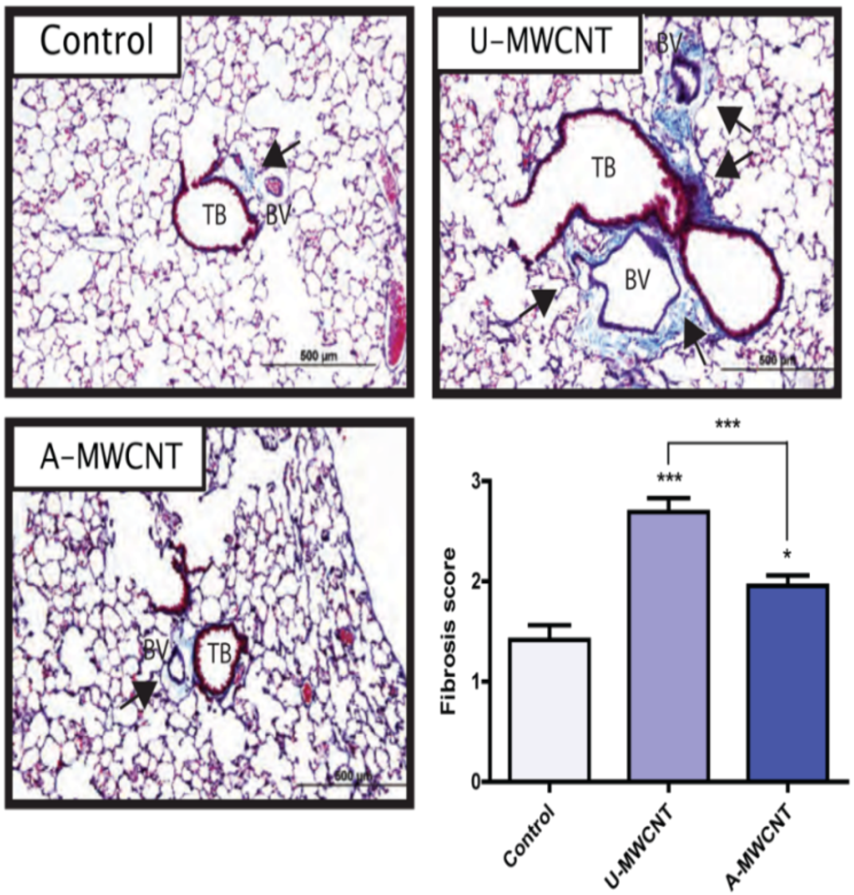

Dandley et al., 2016 Particle & Fibre Tox. 13(1):29.
Taylor et al., 2014 Plos One. 9(9):e106870.

# Atomic Layer Deposition (ALD) Coating with $Al_2O_3$ Reduces MWCNT-induced Lung Fibrosis in Mice

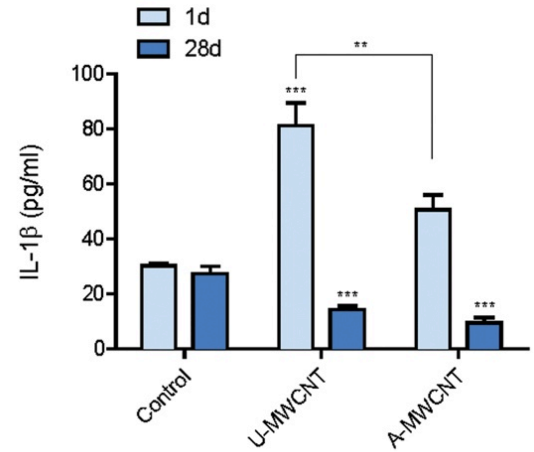
Macrophage uptake

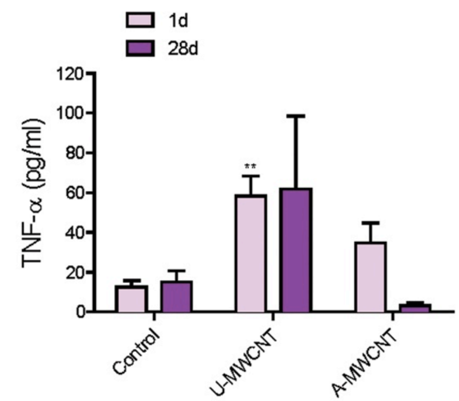



Lung Pathology at 21 days after 2 mg/kg body weight oropharyngeal aspiration




Fibrogenic Cytokines in Broncho-Alveolar Lavage Fluid (BALF)



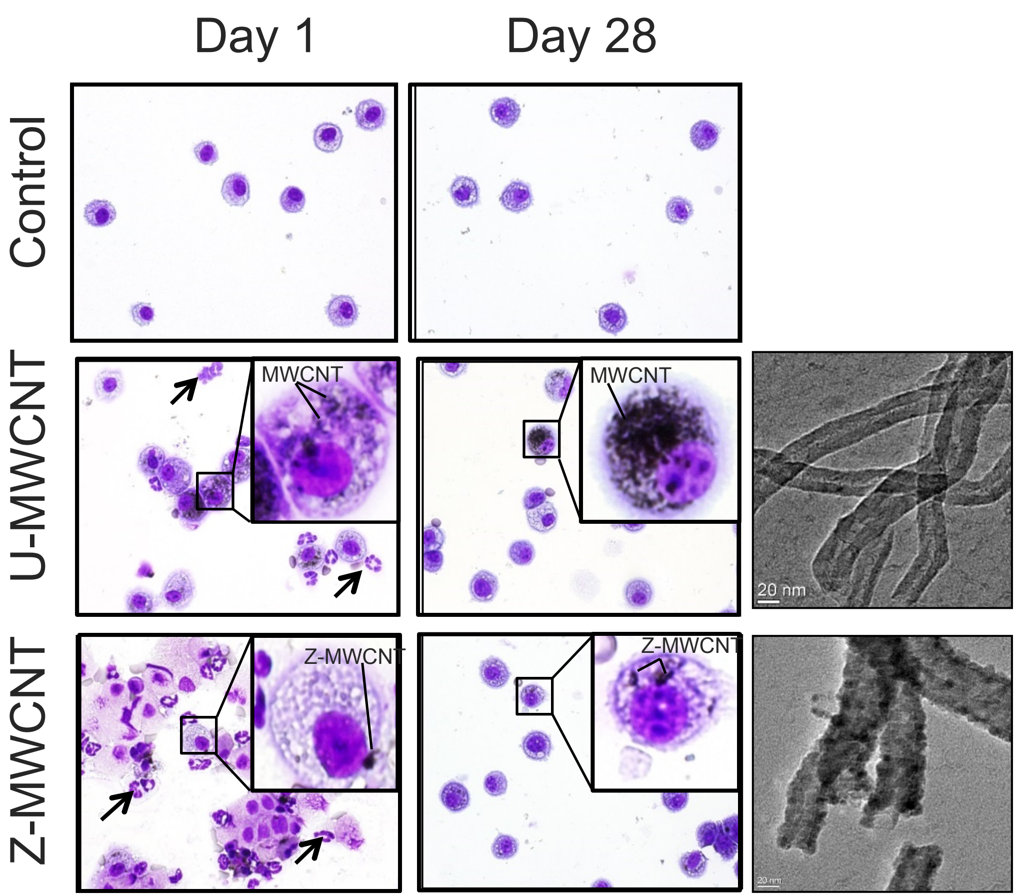



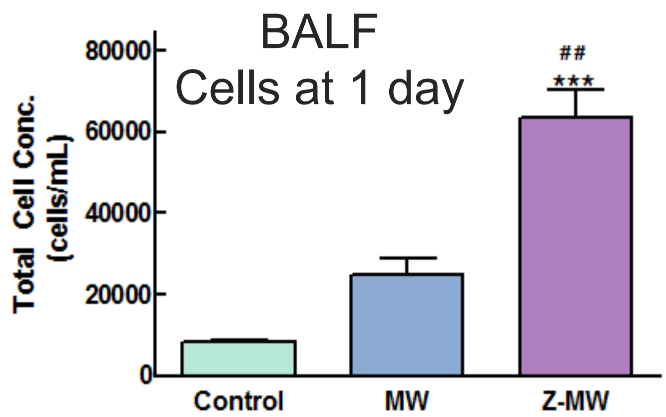

Taylor et al., PLoS One. 2014 Sep 12;9(9):e106870.

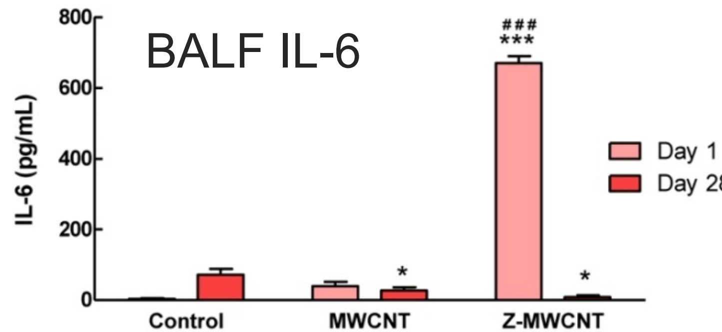
NC STATE UNIVERSITY

# ALD Coating of MWCNT with ZnO Causes Acute Lung and Systemic Inflammatory Responses in Mice

Inflammatory cells in BALF after 2 mg/kg body weight oropharyngeal aspiration

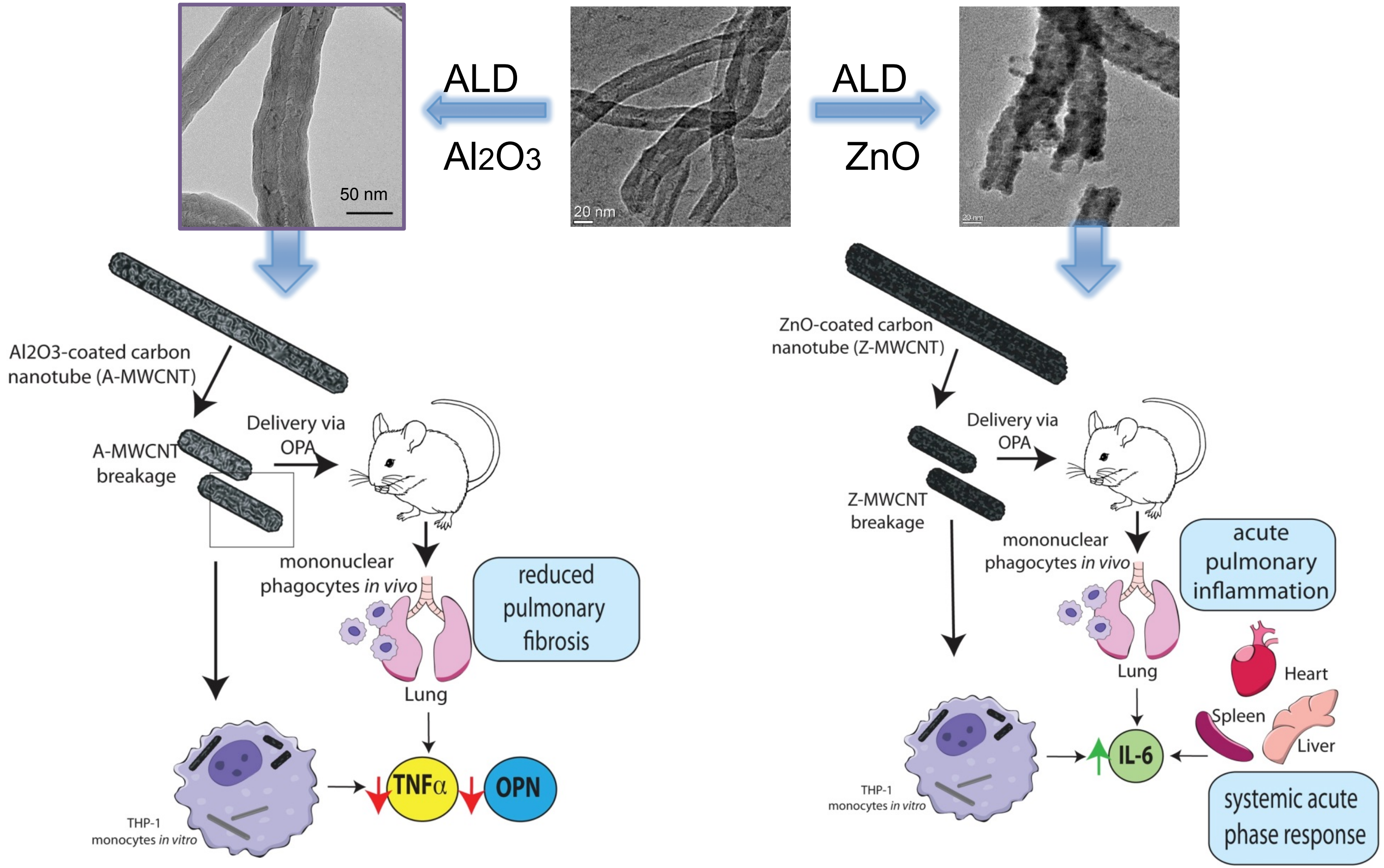


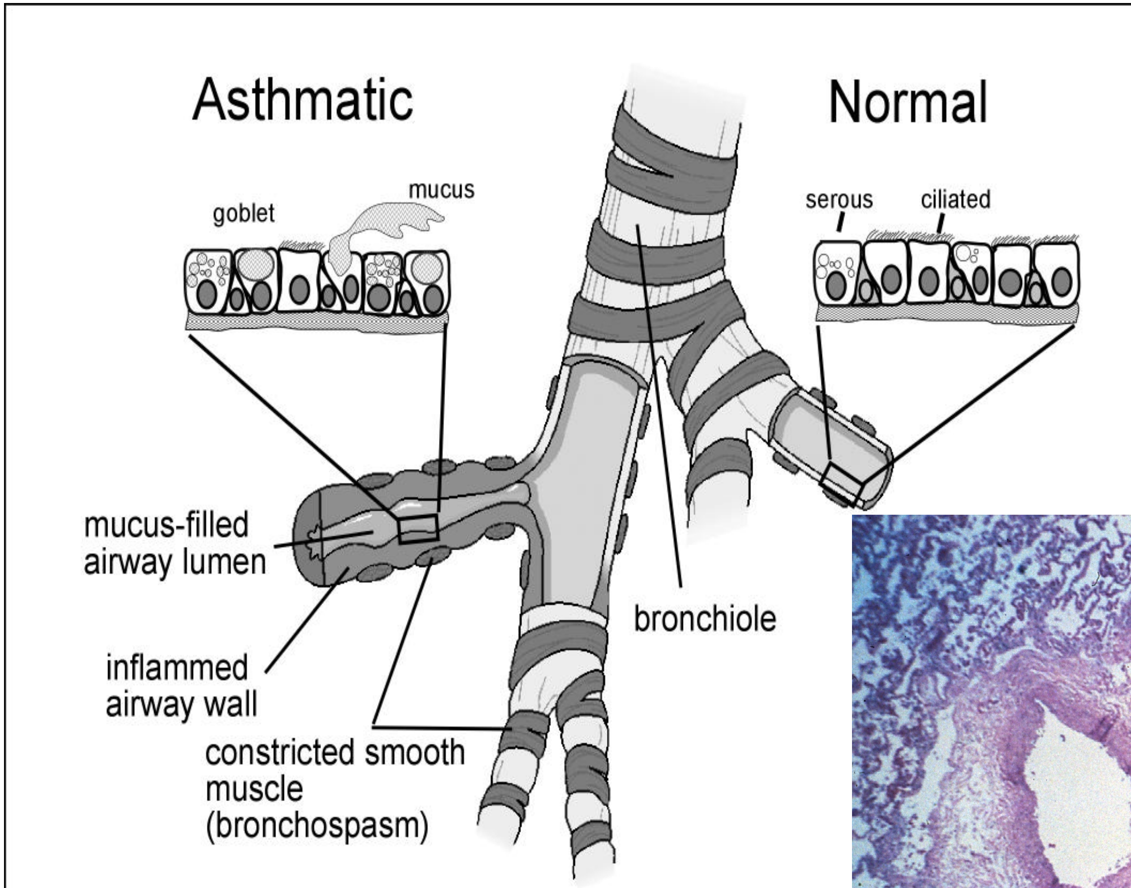

Dandley et al., 2016 Particle & Fibre Tox. 13(1):29.

# Consequences of Atomic Layer Deposition (ALD) Coating of MWCNTs on Disease Outcome

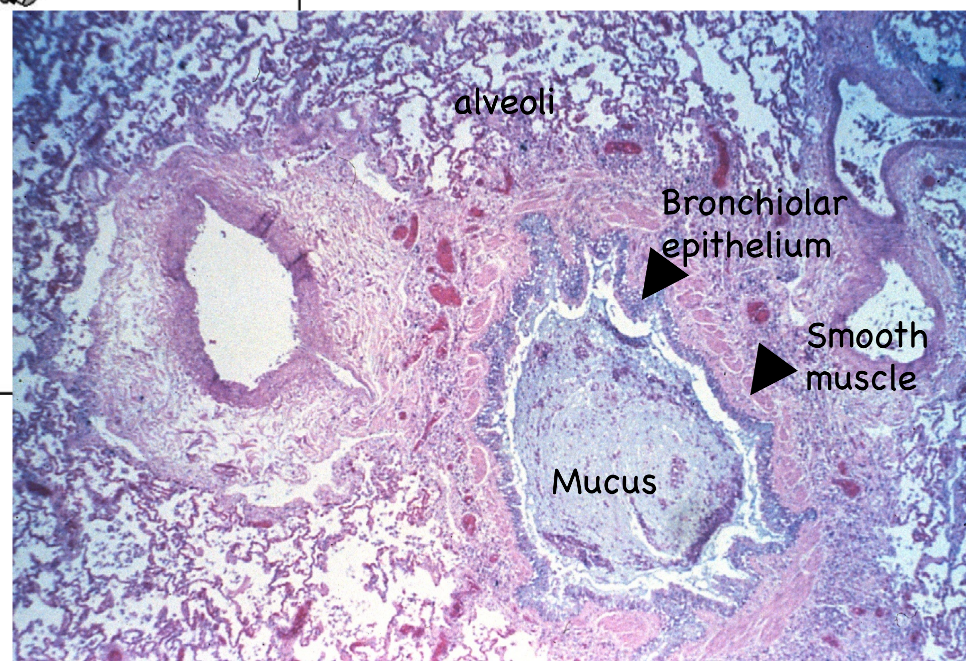


NC STATE UNIVERSITY

# Pathogenesis of Asthma




**Airway Remodeling**
- Goblet cell hyperplasia
- Airway fibrosis
- Smooth muscle thickening
- Eosinophilic lung inflammation
- Increased serum IgE



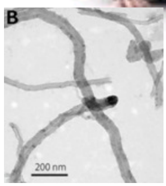

**Caused by Immune Sensitization**
- House dust mite allergen
- Cockroach allergen
- endotoxin

*Bonner JC. Respiratory Toxicology. In Molecular & Biochemical Toxicology, 4th Ed. Smart & Hodgson, 2008*

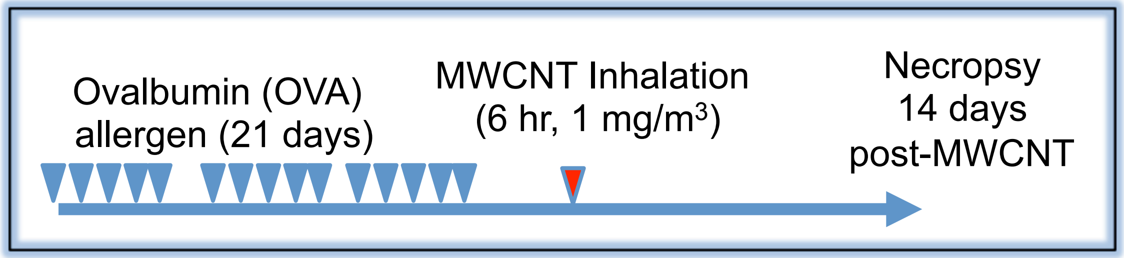
NC STATE UNIVERSITY

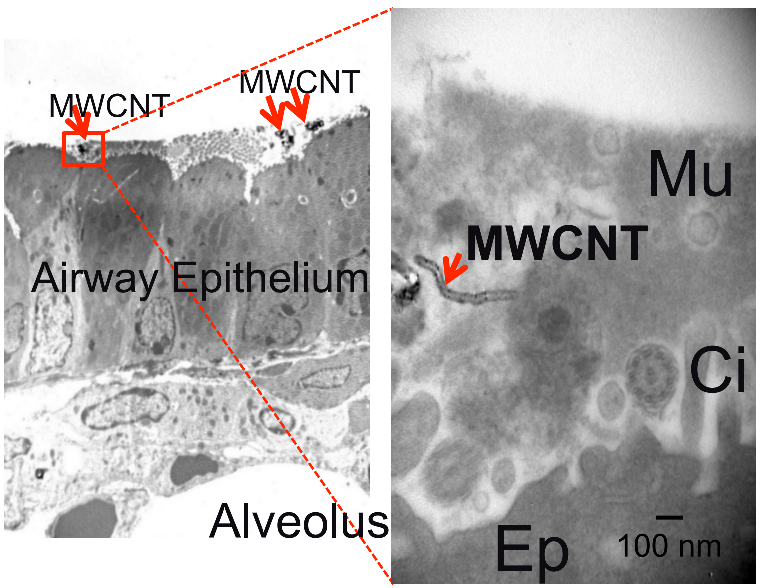
# Exacerbation of Allergen-Induced Airway Fibrosis in Mice by Inhaled Multi-Walled Carbon Nanotubes

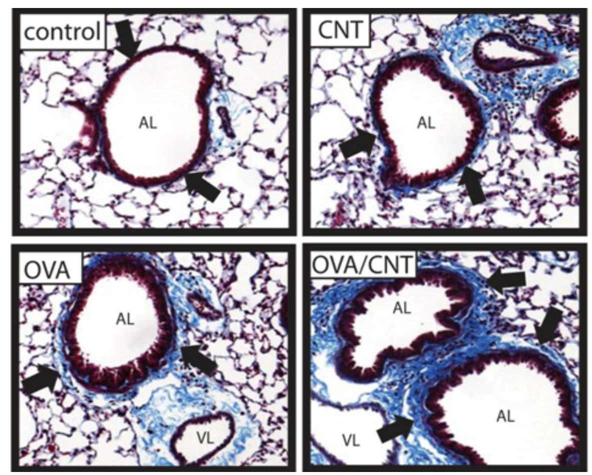
C57BL6 mice

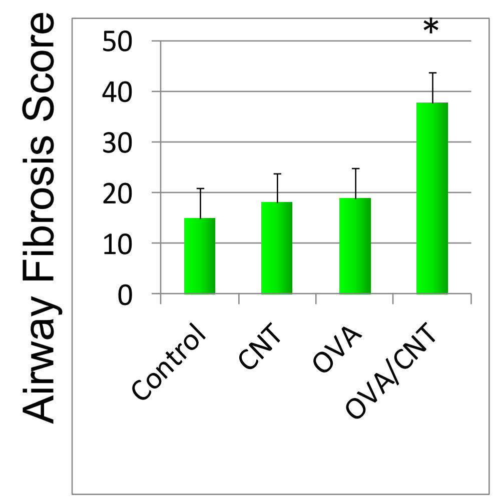



MWCNT (Helix, Inc.)



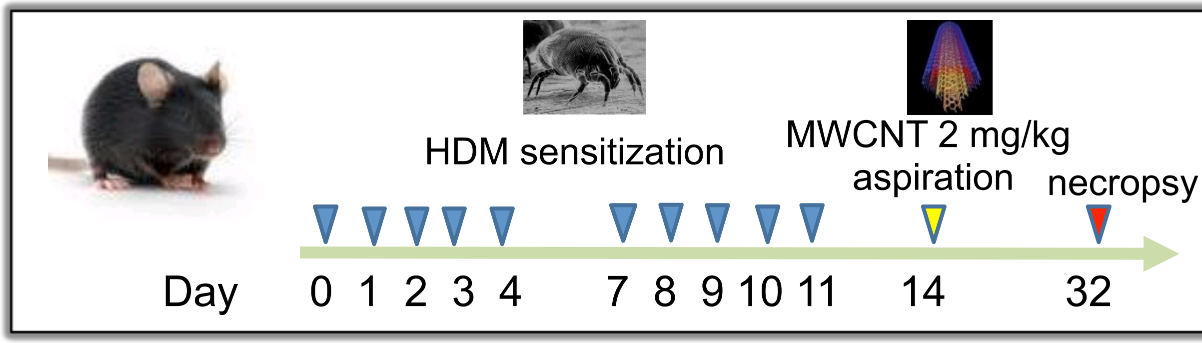



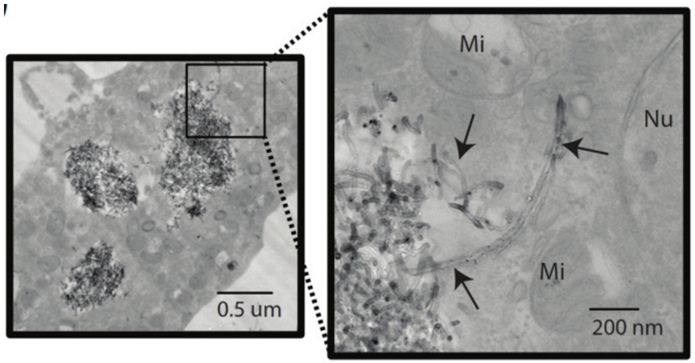



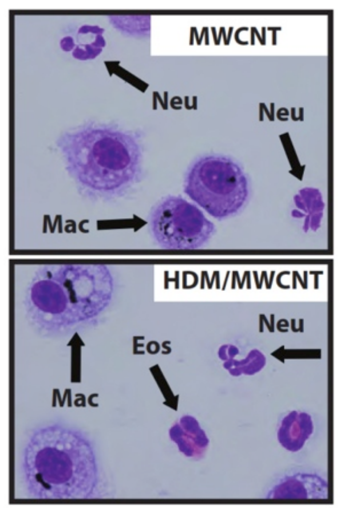

Ryman-Rasmussen *et al. AJRCMB 40:349-358, 2009*

# Exacerbation of House Dust Mite (HDM) Allergen-Induced Airway Fibrosis by MWCNT delivered via Oropharyngeal Aspiration

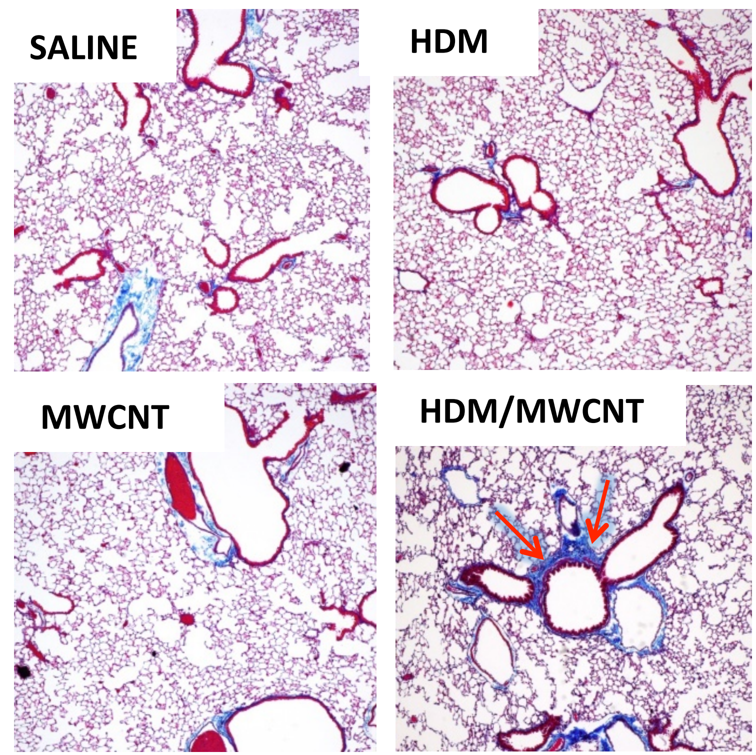



Macrophage uptake of MWCNT (Cheaptubes, Inc.)




Lung Inflammatory Cells in BALF




Pathology (Masson's trichrome stain)

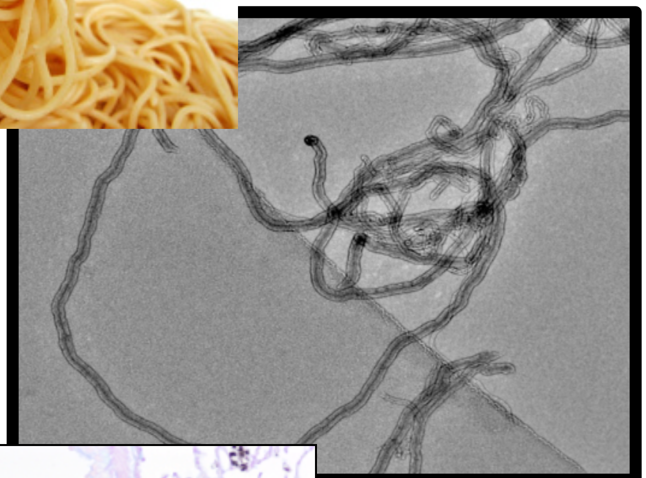
Shipkowski et al., PLoS One. 2015 Jun 19;10(6):e0128888.

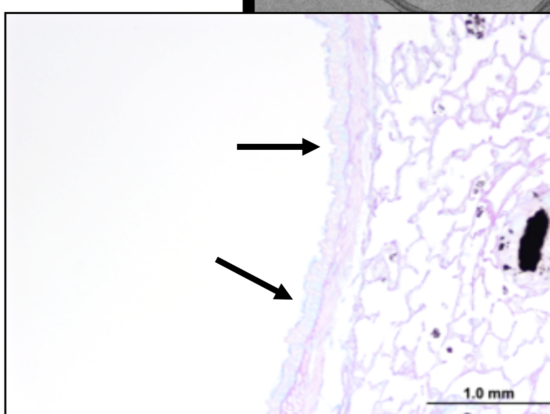
NC STATE UNIVERSITY

# Direct Asthma-like Effects of Rod-like MWCNTs

Cooked sphaghetti

Tangled (Helix)



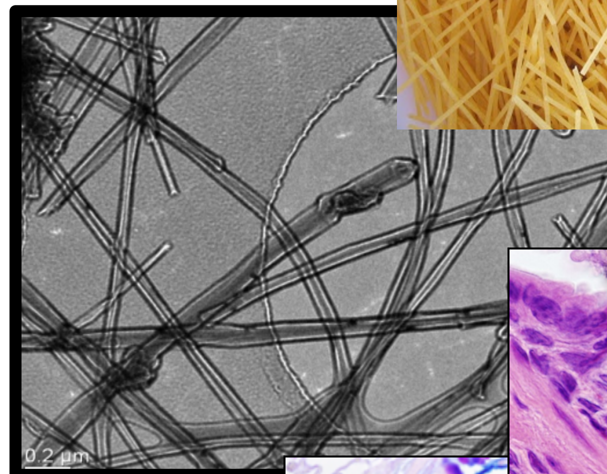



normal mucociliary airway epithelium

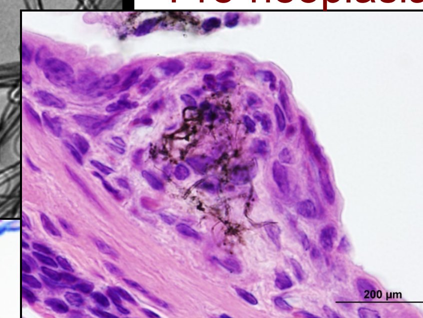
Rod-like (Mitsui-7)

Group 2B
Possible Carcinogen

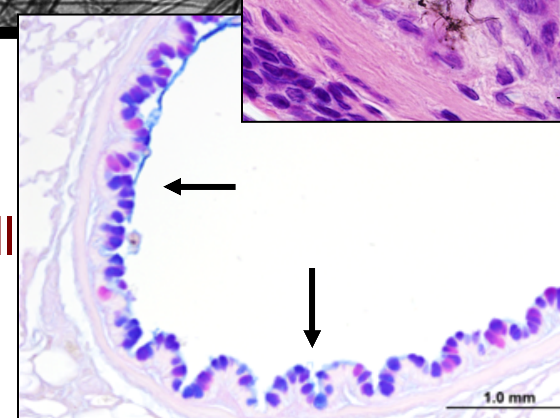
Uncooked sphaghetti




Airway Pre-neoplasia

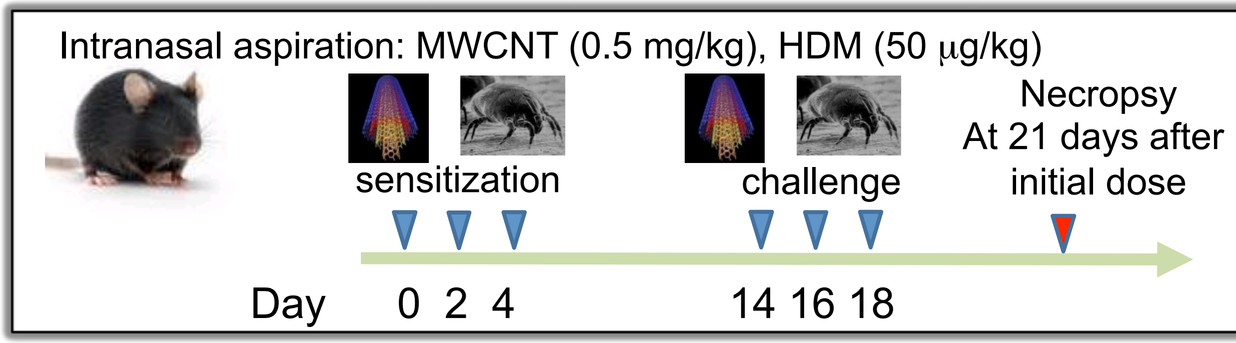


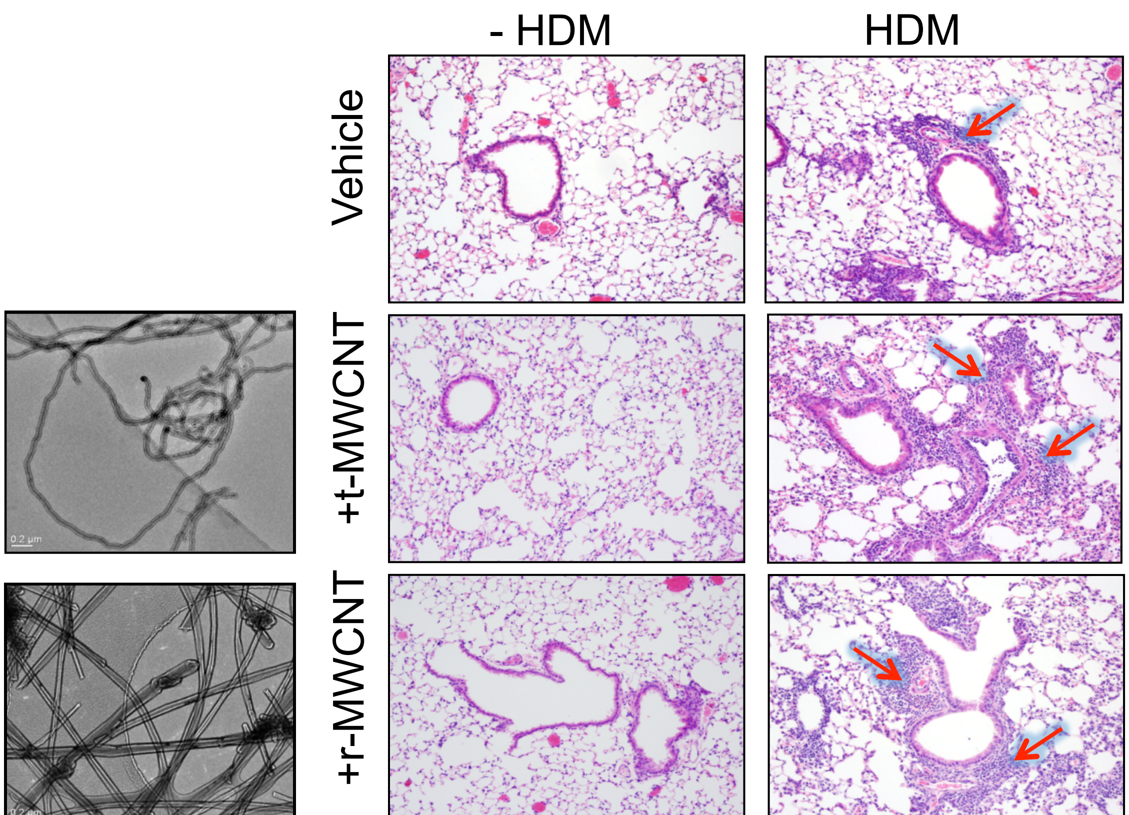

mucous cell metaplasia

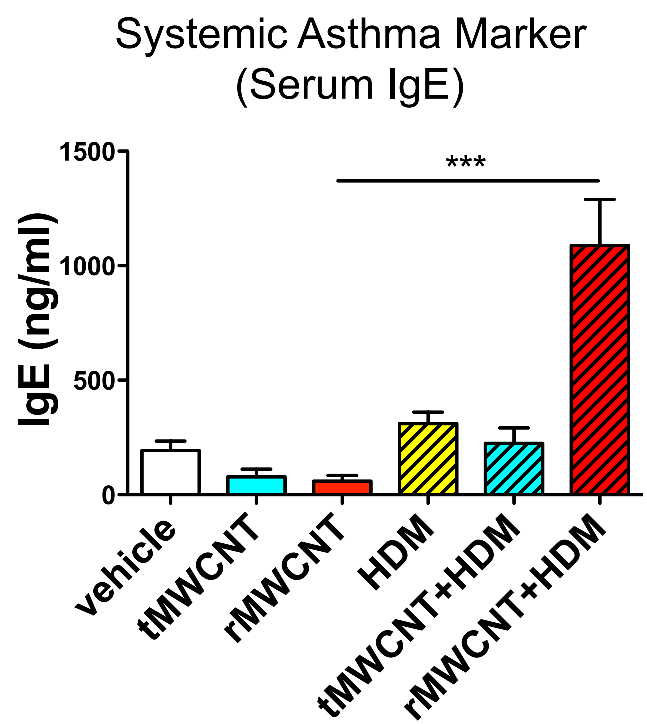


NC STATE UNIVERSITY

# Adjuvant Effect of Tangled (t)-MWCNT or Rigid (r)-MWCNT in House Dust Mite (HDM)-Induced Lung Inflammation in Mice

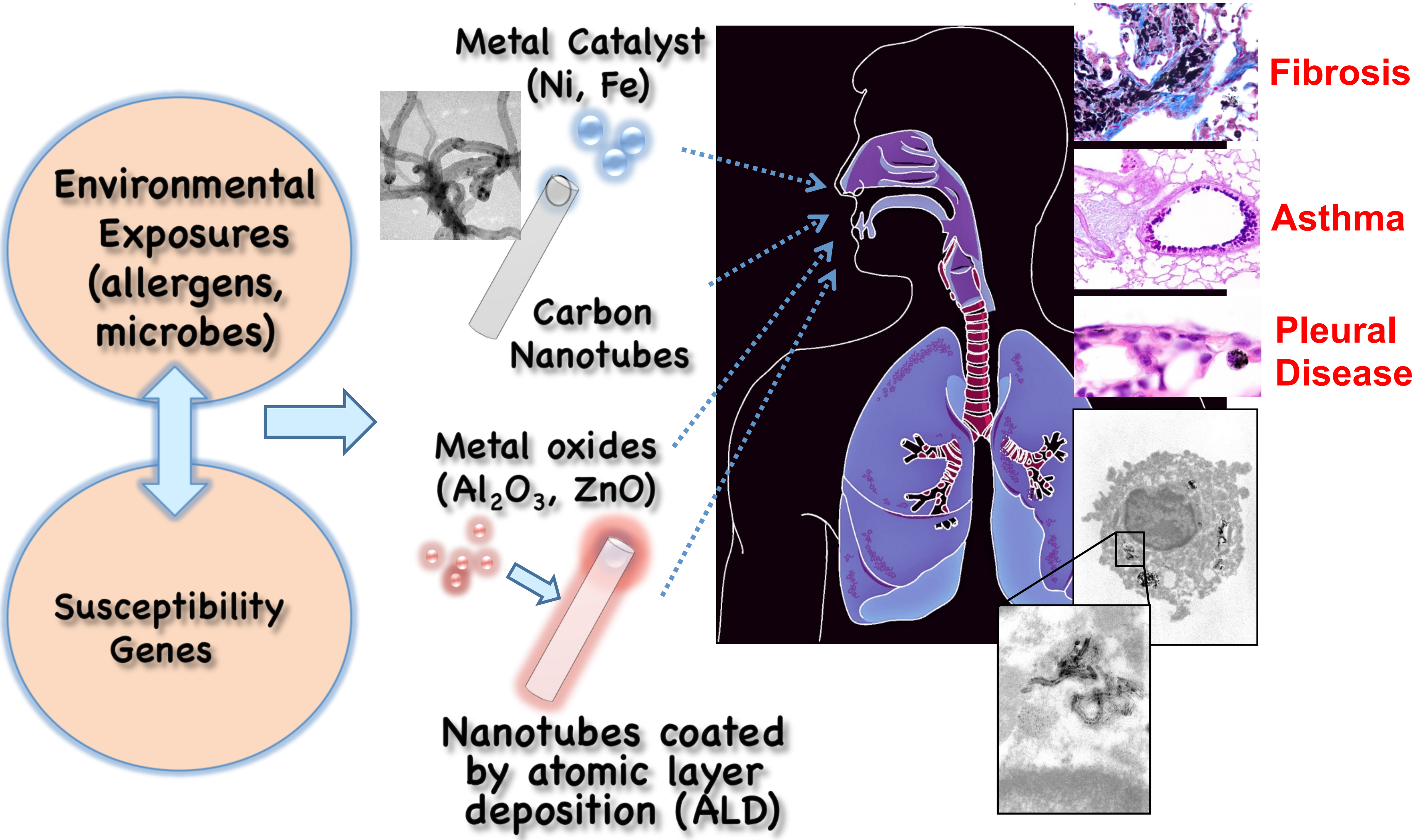


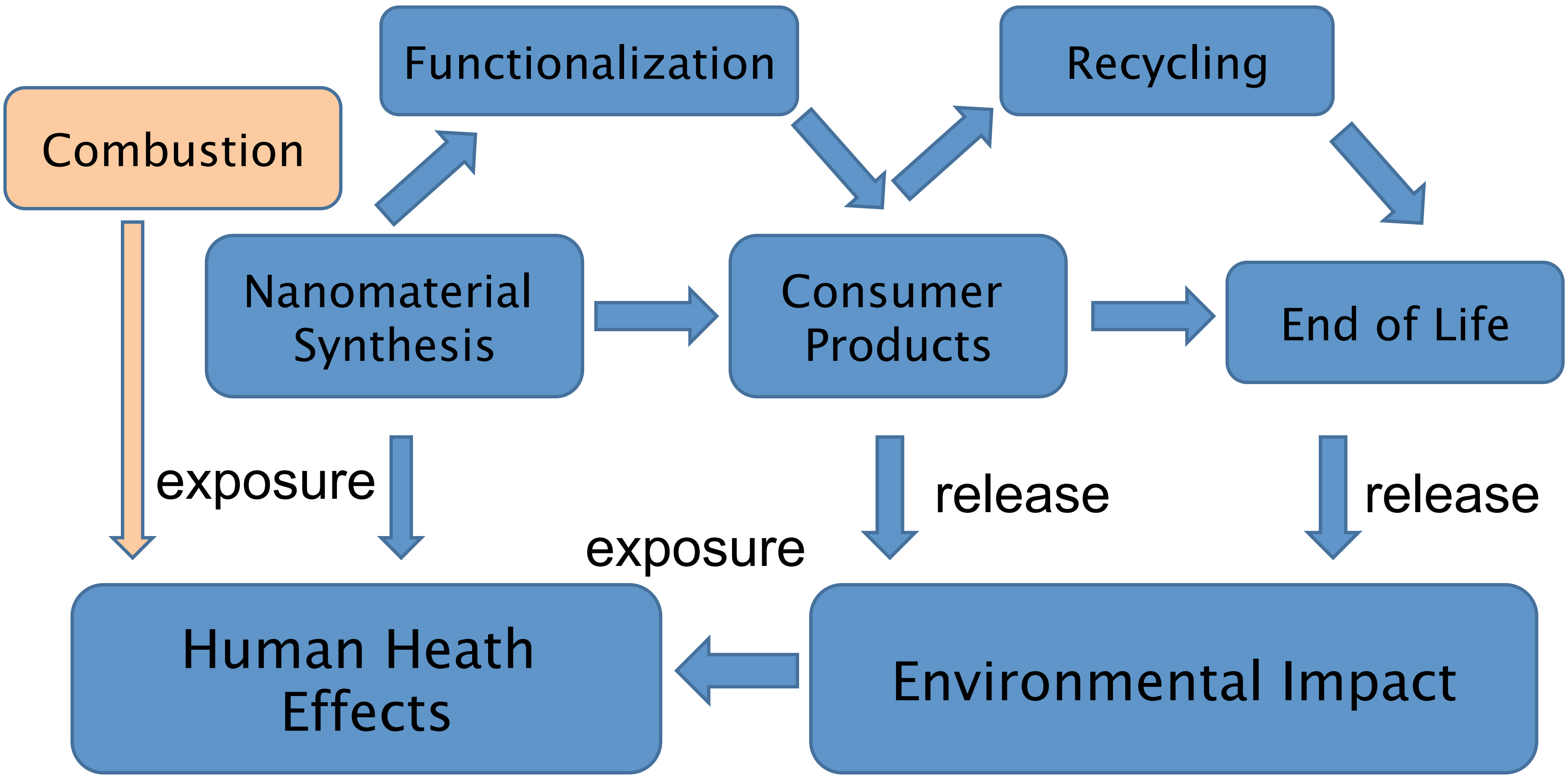
NC STATE UNIVERSITY

# Gene-Environment Interactions in Susceptibility to Complex Mixtures of ENMs



NC STATE UNIVERSITY

# HEALTH AND ENVIRONMENTAL HAZARD ASSESSMENTS OF NANOMATERIALS ALONG THEIR LIFECYCLE



NC STATE UNIVERSITY

# Acknowledgements

**NCSU Toxicology**
Lexie Taylor
Katherine Duke
Mark Ihrie
Gina Hilton
Michael Bereman
Kelly Shipkowski
Brian Sayers
Elizabeth Thompson
Ellen Glista-Baker
Jessica Ryman-Rasmussen

**NCSU Chemical & Biomolecular Engineering**
Gregory Parsons
Christina McClure
Erinn Dandley

**East Carolina University**
Dianne Walters
Chris Wingard
Kym Gowdy

**The Hamner Institutes**
Mel Andersen
Jimmy Mangum
Brian Wong
Rusty Thomas

## Funding

**SIINN Program**
Dominique Lison
Sybille van den Brule
Otto Creutzenberg
Christina Ziemann
Simion Simon
Flaviu Turcu

**NIEHS NanoConsortium**
Andrij Holian
Kent Pinkerton
Jack Harkema
Terrance Kavanagh
Jared Brown
Alison Elder
Gunter Oberdorster
Andre Nel
Dale Porter
Vince Castranova

**NIEHS Intramural**
Stavros Garantziotis
Salik Hussain
Mark Cesta

**Bonner Lab Contact Information**
jcbonner@ncsu.edu
**http://bonnerlab.wordpress.ncsu.edu/**