Mechanisms of Susceptibility to Carbon Nanotube Lung Disease

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**Carbon Nanotubes**

**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)

Carbon Nanotube Synthesis
- Purification
- Functionalization
- Toxicity of Modified Nanomaterial

Metal Catalyst (Ni, Fe)
Metal oxides (Al₂O₃, ZnO)

Nanotubes coated by atomic layer deposition (ALD)

Fibrosis
Asthma
Pleural Disease
Defining the Relative Risk of Carbon Nanotubes

No or low risk

Healthy Lung

Nanotubes as a single type?

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

Asbestosis Lung

Nanotubes as a diverse class?
“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)”

Physico-Chemical Characteristics that Predict Carbon Nanotube Toxicity

Helix MWCNT

Mitsui-7 MWCNT

Workplace Exposure to Carbon Nanotubes

Eight MWCNT manufacturing facilities evaluated; exposures ranged from non-detectable samples to 80 µg/m³

NIOSH recommended exposure limit (REL) of 1 µg/m³ 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.
Relative Human Health Effects: Engineered Carbon Nanotubes (CNTs) vs Combustion-Derived CNTs

Engineered


Combustion-Derived

Inhalation of Carbon Nanotubes in Mice

Nose-only Inhalation setup

TEM Grid

100 nm

High-dose, nose-only
6 hr, 1 day, (1 or 30 mg/m³)
1 wk – 6 mth

low-dose, whole body
6 hr, 5 day/wk, 30 days (0.06, 1, or 0.6 mg/m³)
3 – 30 dy

Inhaled Carbon Nanotubes in Mouse Lung Alveolar Region

Epithelium

Alveolus

MWCNT

100 nm

Alveolus

Macrophage
Acute Pleural Inflammation in Mice after MWCNT Inhalation

Serial sections at 24 hrs

MWCNT Persistence at Pleura after 3 Months

mesothelium

macrophage

nanotubes

lymphocyte

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

Uncoated MWCNT (Helix, Inc.)

Al₂O₃-Coated (A-MWCNT)

ZnO-coated (Z-MWCNT)

Atomic Layer Deposition

1) Introduce diethylzinc to OH surface
2) Purge reactor with nitrogen
3) Introduce water to the reactor
4) Purge reactor with nitrogen

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 Al₂O₃ Reduces MWCNT-induced Lung Fibrosis in Mice


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

Macrophage uptake

Fibrogenic Cytokines in Broncho-Alveolar Lavage Fluid (BALF)

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

Mucus cell metaplasia

ALD \( \text{Al}_2\text{O}_3 \)

ALD \( \text{ZnO} \)

Al\(_2\)O\(_3\)-coated carbon nanotube (A-MWCNT)

A-MWCNT breakage

Delivery via OPA

mononuclear phagocytes in vivo

reduced pulmonary fibrosis

Lung

THP-1 monocytes in vitro

TNF\(\alpha\)

OPN

ZnO-coated carbon nanotube (Z-MWCNT)

Z-MWCNT breakage

Delivery via OPA

mononuclear phagocytes in vivo

Lung

THP-1 monocytes in vitro

IL-6

systemic acute phase response

acute pulmonary inflammation
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
Exacerbation of Allergen-Induced Airway Fibrosis in Mice by Inhaled Multi-Walled Carbon Nanotubes

C57BL6 mice

MWCNT (Helix, Inc.)

Ovalbumin (OVA) allergen (21 days)

MWCNT Inhalation (6 hr, 1 mg/m$^3$)

Necropsy 14 days post-MWCNT

Airway Epithelium

Alveolus

MWCNT

Mu

Ci

100 nm

Airway Fibrosis Score

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)

Direct Asthma-like Effects of Rod-like MWCNTs

Tangled (Helix)

Rod-like (Mitsui-7)
Group 2B
Possible Carcinogen

normal mucociliary airway epithelium

mucous cell metaplasia

Cooked sphaghetti

Uncooked sphaghetti

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

Intranasal aspiration: MWCNT (0.5 mg/kg), HDM (50 µg/kg)

Necropsy At 21 days after initial dose

Day 0 2 4 14 16 18

Vehicle

-HDM

+HDM

Systemic Asthma Marker (Serum IgE)

IgE (ng/ml)

<table>
<thead>
<tr>
<th></th>
<th>vehicle</th>
<th>tMWCNT</th>
<th>rMWCNT</th>
<th>HDM</th>
<th>tMWCNT+HDM</th>
<th>rMWCNT+HDM</th>
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<tbody>
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<td>IgE (ng/ml)</td>
<td>500</td>
<td>1000</td>
<td>1500</td>
<td>2000</td>
<td>3000</td>
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Gene-Environment Interactions in Susceptibility to Complex Mixtures of ENMs

Environmental Exposures (allergens, microbes)

Susceptibility Genes

Metal Catalyst (Ni, Fe)

Carbon Nanotubes

Metal oxides ($\text{Al}_2\text{O}_3$, ZnO)

Nanotubes coated by atomic layer deposition (ALD)

Fibrosis

Asthma

Pleural Disease
HEALTH AND ENVIRONMENTAL HAZARD ASSESSMENTS OF NANOMATERIALS ALONG THEIR LIFECYCLE

Combustion

Nanomaterial Synthesis

Functionalization

Consumer Products

Recycling

End of Life

exposure

Human Heath Effects

release

exposure

release

Environmental Impact
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