Progress Towards Understanding the Health Effects of Carbon Nanotubes

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Carbon Nanotubes

Growing a Forest of Carbon Nanotubes

Scanning EM of Carbon Nanotubes Synthesized by Chemical Vapor Deposition
(Courtesy of Dr. Gregory Parsons, Department of Chemical & Biomolecular Engineering, North Carolina State University)
Uses of Carbon Nanotubes

- Nanoscale electronics & Energy cells
- Medicine
- Tissue Engineering
- Light-weight, super-strong material
- Future Applications

- Nanotube field effect transistor
- Cancer Therapeutics
- Artificial muscle
- Bike Frame
- Air Frame
- Military Apps

- Space elevator
- Artificial muscle suit
Size and Shape Predict Toxicity

Macrophages

Asbestos

Lung Macrophage
12.4 μm diameter

Lung Diseases and Carbon Nanotubes

- Asthma
- Fibrosis
- Pleural Disease
Topics to be Discussed

- The evidence for carbon nanotube (CNT)-induced lung fibrosis in animals and predictions for human exposure.
- The evidence for CNT-induced mesothelioma and implications for human exposure.
- Susceptibility to CNTs and exacerbation of pre-existing disease
- Systemic effects of inhaled CNTs
- Consequences of CNT Functionalization on Toxicity
- Progress with consortium testing of CNTs
Occupational and Environmental Exposures to Carbon Nanotubes and Human Health Risks

Mode of Action in Biological Systems

No human disease so far… but animal models using inhalation exposure indicate significant risk

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Occupational Exposure to Carbon Nanotubes and Nanofibers

Regulatory Recommendations
(1) reviews the animal and other toxicological data relevant to assessing the potential non-malignant adverse respiratory effects of CNT and CNF,

(2) provides a quantitative risk assessment based on animal dose-response data,

(3) proposes a recommended exposure limit (REL) of 1 μg/m³ elemental carbon as a respirable mass 8-hour time-weighted average (TWA) concentration, and

(4) describes strategies for controlling workplace exposures and implementing a medical surveillance program. The NIOSH REL is expected to reduce the risk for pulmonary inflammation and fibrosis. However, because of some residual risk at the REL and uncertainty concerning chronic health effects, including whether some types of CNTs may be carcinogenic, continued efforts should be made to reduce exposures as much as possible.

http://www.cdc.gov/niosh/docs/2013-145/
Carbon Nanotubes and Lung Cancer – The NIOSH Study

(1) The study was designed to investigate whether MWCNT have the potential to initiate or promote cancer.

(2) Mice receiving both an initiator chemical plus inhalation exposure to MWCNT were significantly more likely to develop tumors (90% incidence) and have more tumors than mice receiving the initiator chemical alone.

(3) These results indicate that MWCNT can increase the risk of cancer in mice exposed to a known carcinogen.

(4) The study did not indicate that MWCNTs alone cause cancer in mice.


http://www.cdc.gov/niosh/docs/2013-145/
Occupational Exposure Risks During Synthesis and Functionalization of Carbon Nanotubes

Synthesis

Post-synthesis Functionalization (Atomic Layer Deposition)

Metal Catalyst (Ni, Fe)

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

Modified Nanomaterial (Unknown Consequences of Functionalization)

Carbon Nanotubes

Thin Film Coated Nanotubes

Metal oxides ($\text{Al}_2\text{O}_3, \text{ZnO}$)
Occupational Exposure to CNTs and Lung Disease

The Journey Begins 10 Years Ago:

Single-Walled Carbon Nanotubes Cause Granulomas in the Lung of Rats


Early studies such as this one showed granuloma formation but it was unclear whether this pathology was due to non-specific effects of CNTs. This investigation illustrated the issue of CNT aggregation, a problem that would later be overcome by the use of dispersion medium containing surfactants.
SWCNT increased alveolar wall thickness accompanied by increased levels of the pro-fibrogenic mediator TGF-β1.

“SWCNT-induced fibrotic lesions in rats were not due to a non-specific carbon nanoparticle effect.”
Some early Inhalation Studies with Mice Showed Little Pro-fibrogenic Activity of Carbon Nanotubes


“No significant lung inflammation or fibrosis in response to inhaled MWCNTs, but splenic immune response with elevated IL-6 and IL-10”

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

“No significant lung fibrosis seen 14 days after a 6 hr inhalation high dose of 30 mg/m³, unless mice were pre-exposed to allergen, then significant airway fibrosis was observed”

The lack of fibrogenic reactions in the these studies could due to relatively short time frame [2 weeks] after exposure or could be due to the type of MWCNTs used.
Aspiration or Inhalation Exposure to MWCNTs Produces Progressive Fibrosis in Mice

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

“MWCNT exposure [in C57BL6 mice] caused rapid [dose & time-dependent] development of pulmonary fibrosis by 7 days post-exposure, that granulomatous inflammation persisted throughout the 56-day post-exposure period”.

Mercer et al., 2013 Part. & Fibre Tox. 10:33

“Inhalation exposure to MWCNT produced a fibrotic response that was found to develop and persist out to 336 days after exposure, which is significantly longer than examined in prior bolus aspiration studies.”

“Singlet MWCNTs… retained within the alveolar septa produce a progressive fibrotic reaction in the lungs”
Inhalation Studies are Critical as they Model More Realistic “Real World” Exposures

Inhaled Carbon Nanotubes at the Muco-ciliary Zone in the Airway of a C57BL6 Mice
Inhaled Carbon Nanotubes in Mouse Lung Alveolar Region

- Alveolus
- Epithelium
- MWCNT
- 100 nm
- Macrophage

Scale bar: 100 nm
Clearance & Degradation of Carbon Nanotubes in mice after Inhalation Exposure

- CNT removal from the lung is primarily through macrophage-mediated mucociliary clearance and lymphatic drainage.

- Degradation is mediated by neutrophil myeloperoxidase (MPO) to some extent (Kagan et al., 2010 Nat. Nanotech. 5:354). Also, MPO-deficient mice have impaired clearance and enhanced lung inflammation to CNTs (Shvedova et al. 2012 Plos One 7(3): e30923.)

- Nevertheless, some CNTs are biopersistent in lung cells & tissues for months after inhalation exposure (Ryman-Rasmussen et al. 2009 Nature Nanotech Nov;4(11):747-51.)
Interaction of CNTs with Intracellular Components

- **SWCNT bridges between macrophages could be due to interaction with cytoskeletal components** (Mangum et al., 2006 Part. Fibre Tox. 3:15)

- **SWCNT also disrupt mitotic spindle formation in epithelial cells** (Sargent et al. 2012, Mutat. Res. 745:28-37).

“Unique interactions of carbon nanotubes with cytoskeletal or other subcellular structures could impede or disrupt critical processes such as cell motility and division.”
Workplace Exposure to Carbon Nanotubes

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

“**In these 8 MWCNT facilities, exposures ranged from non-detectable samples to 79.6 μg/m³**”

“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.”
Susceptibility Factors in Carbon Nanotube-Induced Respiratory Disease

Susceptibility factors:
- Pre-existing disease (asthma, COPD)
- Susceptibility genes
- Pre-existing bacterial or viral infection

Pathologic or physiologic change:
- Reduced mucociliary clearance
- Airway, interstitial or pleural fibrosis
- Enhanced inflammation

Susceptibility to Carbon Nanotubes due to Allergen or Bacterial Pre-Exposure

Allergen-Induced Susceptibility


Bacterial-Induced Susceptibility


“*These studies indicated that carbon nanotubes would be most hazardous to individuals with pre-existing disease, but this is also true for some other nano-sized particles, including ultrafine diesel exhaust particulates (which exacerbate asthma in humans)*”
“Most rodent studies show that CNTs cause neutrophilic lung inflammation, but also modulate allergen-induced eosinophilic inflammation and worsen allergen-induced responses”
Exacerbation of Pre-Existing Allergic Airway Inflammation by Carbon Nanotubes

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

OVA (21 days) → Nanotube Inhalation (30 mg/m³ for 6 h) → Collect Lung Tissues (14 days)

Airway Collagen Thickness Score

Control  CNT  OVA  OVA/CNT

Ryman-Rasmussen et al. AJRCMB 40:349-358, 2009
Carbon Nanotubes and Innate Immunity: The Inflammasome

Palomaki et al., 2011 Long, needle-like carbon nanotubes and asbestos activate the NLRP3 inflammasome through a similar mechanism. ACS Nano 5:6861.

“Long rigid tubes activate inflammasome and IL-1β release, but activation is also dependent on ROS and Cathepsin B”

Wang et al., 2011 Dispersal state of multiwalled carbon nanotubes elicits profibrogenic cellular responses that correlate with fibrogenesis biomarkers and fibrosis in the murine lung. ACS Nano 5:9772.

“…dispersal state of MWCNTs affects profibrogenic cellular responses [including IL-1β release] that correlate with the extent of pulmonary fibrosis”

Hamilton et al., 2012 NLRP3 Inflammasome Activation in Murine Alveolar Macrophages… Inhal. Tox. 24:995.

“MOA for Ni-contaminated MWCNT was in their ability to disrupt macrophage phagolysosome, which resulted in NLRP3 [inflammasome] activation”

CNTs, like asbestos, activate inflammasomes and this is dependent on length, rigidity, metal catalyst & dispersion state
"Long, rigid CNTs will be retained at the visceral or parietal pleura, whereas short or tangled tubes will be cleared via the Lymphatic system"

"In essence long, rigid CNTs might behave like asbestos"
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)”
“Inhaled CNTs rapidly reach the pleura in mice and stimulate mononuclear cell accumulation on the pleural surface, but these are not mesothelioma and resolve within days after a single inhalation exposure.”

“Repeated inhalation exposures may be needed to determine whether CNTs cause mesothelioma or perhaps a better animal model”
Proposed Mode of Action for CNTs in Pleural Inflammation and Fibrosis in Mice

“Pleural Inflammation and/or pleural fibrosis might be contributory to mesothelioma.”

Carbon Nanotubes and Mesothelioma in p53 KO Mice

- Xu et al., 2013 Mult-walled carbon nanotubes translocate into the pleural cavity and induce visceral mesothelial proliferation in rats. Cancer Sci. 103:2045.

“intrapulmonary administration of multi-walled carbon nanotubes, like asbestos, induced mesothelial proliferation potentially associated with mesothelioma development.”

Nevertheless, whether or not CNTs cause mesothelioma remains controversial & has only been observed in p53^-/+ in the abdomen.
Summary of CNTs and mesothelioma

- Delivery of CNTs into the abdominal cavity of mice by injection show granulomas on the mesothelial lining of the body cavity (Poland et al., 2008 Nature Nano 3, 423)

- Exposure of mice to CNTs by inhalation show migration to the pleura along with pleural inflammation and subpleural fibrosis that resolved (Ryman-Rasmussen et al., 2009 Nature Nano 4, 747).

- CNTs delivered to the lungs of mice cause pleural penetrations (Mercer et al., 2010 Part. Fibre Tox. 7:28).

- Injection of CNTs in the abdominal cavity of p53+/- mice show increased incidence of mesothelioma (Takagi et al., 2012 Cancer Sci. 103, 1440).

- Delivery of CNTs to the lungs of rats via tracheal instillation causes visceral mesothelial cell proliferation similar to that of asbestos (Xu et al., 2012 Cancer Sci 103: 2045).
Systemic Translocation of Nanoparticles

“…non-cationic nanoparticles smaller than ~34 nm in diameter that do not bind serum proteins reach the regional lymph nodes within 30 min”

“Nanoparticles larger than ~34 nm are consistently retained within the lungs."


There is little information available on CNT systemic translocation

From: Kreyling et al., 2010

“…the IL-33/ST2 axis orchestrates adverse pulmonary and cardiovascular responses to an engineered nanomaterial [MWCNT], giving insight into a previously unknown mechanism of toxicity.”

From: Katwa et al., 2012
Carbon Nanotubes and Systemic Effects: Splenic Immunosuppression

Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice

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The potential health effects of inhaling carbon nanotubes are important because of possible exposures in occupational settings. Previously, we have shown mice that have inhaled multiwalled carbon nanotubes have suppressed systemic immune function. Here, we show the mechanisms for this immune suppression. Mice were exposed to 0, 0.3 or 1 mg m\textsuperscript{-3} multiwalled carbon nanotubes for 6 h per day for 14 consecutive days in whole-body inhalation chambers. Only those exposed to a dose of 1 mg m\textsuperscript{-3} presented suppressed immune function; this involved activation of cyclooxygenase enzymes in the spleen in response to a signal from the lungs. Spleen cells from exposed animals partially recovered their immune function when treated with ibuprofen, a drug that blocks the formation of cyclooxygenase enzymes. Knockout mice without cyclooxygenase enzymes were not affected when exposed to multiwalled carbon nanotubes, further confirming the importance of this enzyme in suppression. Proteins from the lungs of exposed mice suppressed the immune function of spleen cells from normal mice, but not those from knockout mice. Our findings suggest that signals from the lung can activate signals in the spleen to suppress the immune function of exposed mice.

“...signals from the lung can activate signals in the spleen to suppress the immune function of exposed mice”

Systemic Translocation of Carbon Nanotubes in Mice After Inhalation Exposure

“Inhaled MWCNT, which deposit in the lungs are transported to parietal pleura, the respiratory musculature, liver, kidney, heart and brain in a singlet form”

“The tracheobronchial lymph nodes contain high levels of MWCNT following exposure and further accumulate over nearly a year to levels that are a significant fraction of the lung burden 1 day post-exposure.”

From: Mercer et al., 2013 Part. Fibre Tox. 10:38.

This study demonstrated the need to address extrapulmonary health effects after inhalation exposure to carbon nanotubes.
Catalysts used in CNT synthesis

Ni/La  Co/Mo  Fe/Al

Carbon Nanotubes

Thin Layer Coatings (e.g., metal oxides)

Outer Shell Additions (e.g., COOH)

It is no longer a simple case of considering carbon nanotube toxicity, but a more complex issue of evaluating a complex nano-mixture.

Hamilton et al., 2013 Effects of MWCNT size, carboxylation, and purification on in vitro and in vivo toxicity, inflammation and lung pathology Part. Fibre Toxicol. 10:57.

“Functionalization by carboxylation completely eliminated the bioactive potential of the MWCNT regardless of size in in vitro testing.”

Li et al., 2013 Surface charge and cellular processing of covalently functionalized multiwall carbon nanotubes determine pulmonary toxicity. ACS Nano 7:2352.

“Compared to pristine MWCNTs, strong cationic PEI-MWCNTs induced significant lung fibrosis, while carboxylation significantly decreased [fibrosis].”

“Surface charge plays an important role in the structure-activity relationships that determine the pro-fibrogenic potential of f-CNTs in the lung.”
Functionalization to Decrease Toxicity

Carbon nanotubes → Purification → Functionalization

Physicochemical change
- Aggregation
- Reduced metal content
- Increased dispersion and biodegradability

Effect on cells or tissues
- Macrophage uptake and inflammasome activation
- Decreased ROS and cellular injury
- Greater bioavailability and cell-specific targeting

Outcome
- Fibrosis and granuloma
- Reduced toxicity
- Therapeutic potential

Toxicity

Predicting the Human Health Effects of ENMs

Interlaboratory Evaluation of in Vitro Cytotoxicity and Inflammatory Responses to Engineered Nanomaterials: The NIEHS Nano GO Consortium

Tian Xia,1 Raymond F. Hamilton Jr.,2 James C. Bonner,3 Edward D. Crandall,4 Alison Elder,5 Farnoosh Fazlollahi,4 Teri A. Girtsman,2 Kwang Kim,4 Somenath Mitra,6 Susana A. Ntim,6 Galya Orr,7 Mani Tagmount,8 Alexia J. Taylor,3 and Frank A. Witzman

Interlaboratory Evaluation of Rodent Pulmonary Responses to Engineered Nanomaterials: The NIEHS Nano GO Consortium

James C. Bonner,1 Rona M. Silva,2 Alexia J. Taylor,1 Jared M. Brown,3 Susana C. Hilderbrand,3 Vincent Castranova,4 Dale Porter,4 Alison Elder,5 Günther Oberdörster,5 Jack R. Harkema,6 Lori A. Bramble,6 Terrance J. Kavanagh,7 Dianne Botta,7 Andre Nel,8 and Kent E. Pinkerton2

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Xia et al., 2013, Environ Health Perspect, 121:683.
Bonner et al., 2013, Environ Health Perspect, 121: 676.
The Consortium Effort: Determining Inter-Laboratory Reproducibility in Mice Exposed to MWCNTs

Bonner et al., 2013, Environ Health Perspect, 121: 676.
“A major goal of the consortium effort was replication and comparability of findings of carbon nanotube toxicity and hazard ranking using a combination of in vivo and in vitro experiments performed by different laboratories across the country using harmonized protocols and well-characterized nanomaterials.”
Predicting Human Health Hazard of ENMs

A Multi-Stakeholder Perspective on the Use of Alternative Test Strategies for Nanomaterial Safety Assessment

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Output of Conference on “Alternative Testing Strategies for Carbon Nanotubes and other Models of Nanomaterial Toxicity.”
UC Center for Environmental Implications of Nanotechnology, UCLA, Los Angeles, CA, January 16-17, 2013.

Nel et al., 2013 ACS Nano, 7:6422.
Summary and Conclusions

- Based on rodent studies CNTs are a probable risk for lung fibrosis in occupational settings, although relative risk depends on the physical and chemical characteristics, including the levels of metal catalysts.

- It remains unknown whether CNTs cause mesothelioma. This is likely due to poor animal models, the infancy of the nanotechnology field and the long latency time for disease development.

- More information is needed about workplace exposures, the types of CNT (including functionalized CNTs) being used in the workplace in order to determine if CNTs are a cancer risk. CNTs alone do not appear to cause lung cancer.

- There is evidence from mouse models that CNTs cause systemic effects (e.g., immunosuppression) by indirect mechanisms and CNTs are capable of escaping the lung to enter lymph nodes and distant organs.

- Consortium Efforts are Valuable Towards Predicting the Health Effects of Nanomaterials and future efforts should be coupled with high through put screening efforts to address the wide variety of functionalized CNTs.
Questions?

Upcoming SOT NTSS Webinar:

Monday, February 10\textsuperscript{th}, 2014 2PM EST

Barbara Harthorn, PhD, Professor and Director, Center for Nanotechnology in Society, University of California Santa Barbara

“Surveying the nanomaterial industry: lessons learned and challenges”