NIOSH CIB:
Occupational Exposure to Carbon Nanotubes and Nanofibers

Summary Of Toxicological Data

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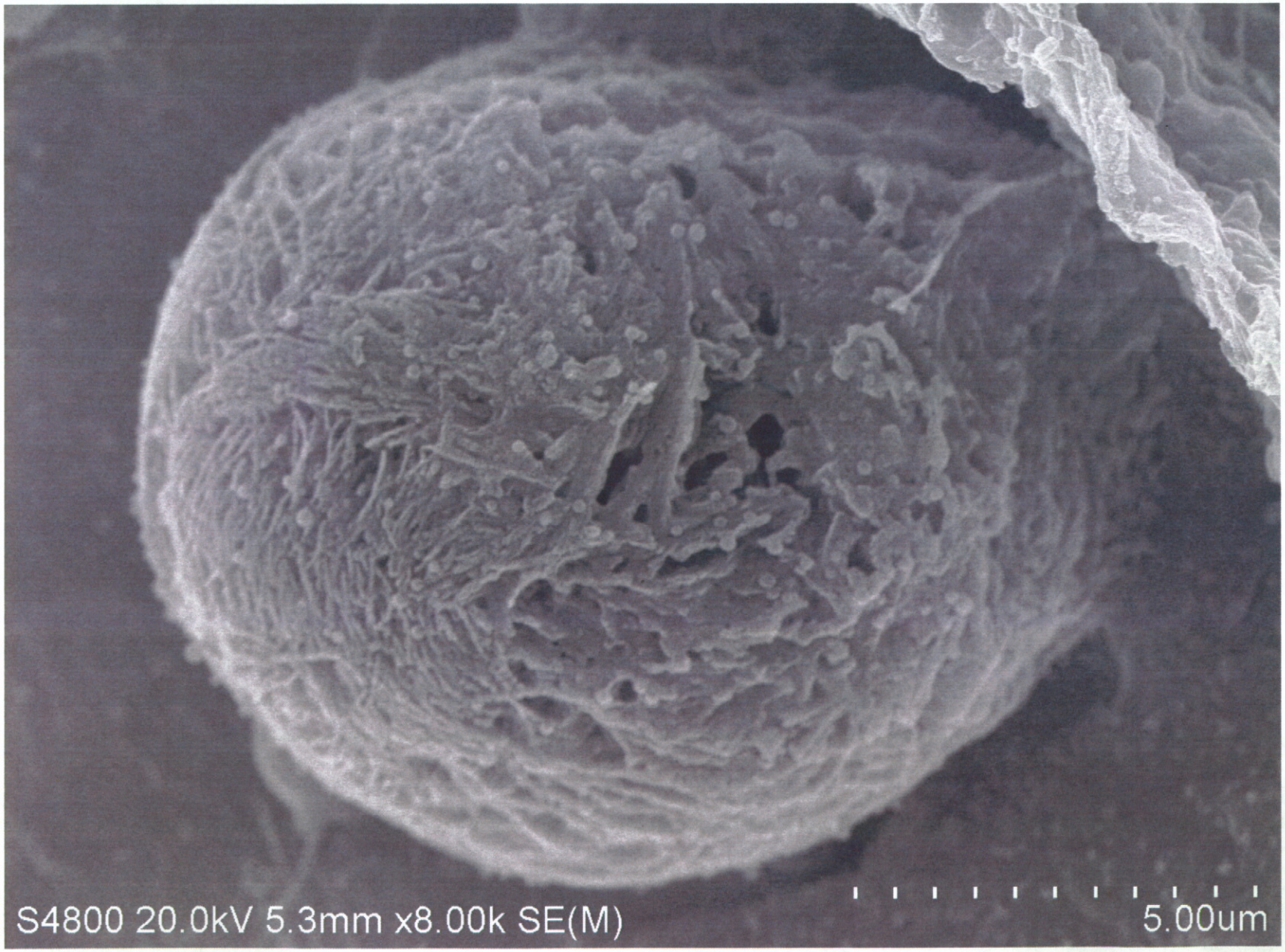
Pulmonary Toxicology Studies Reviewed for the CIB

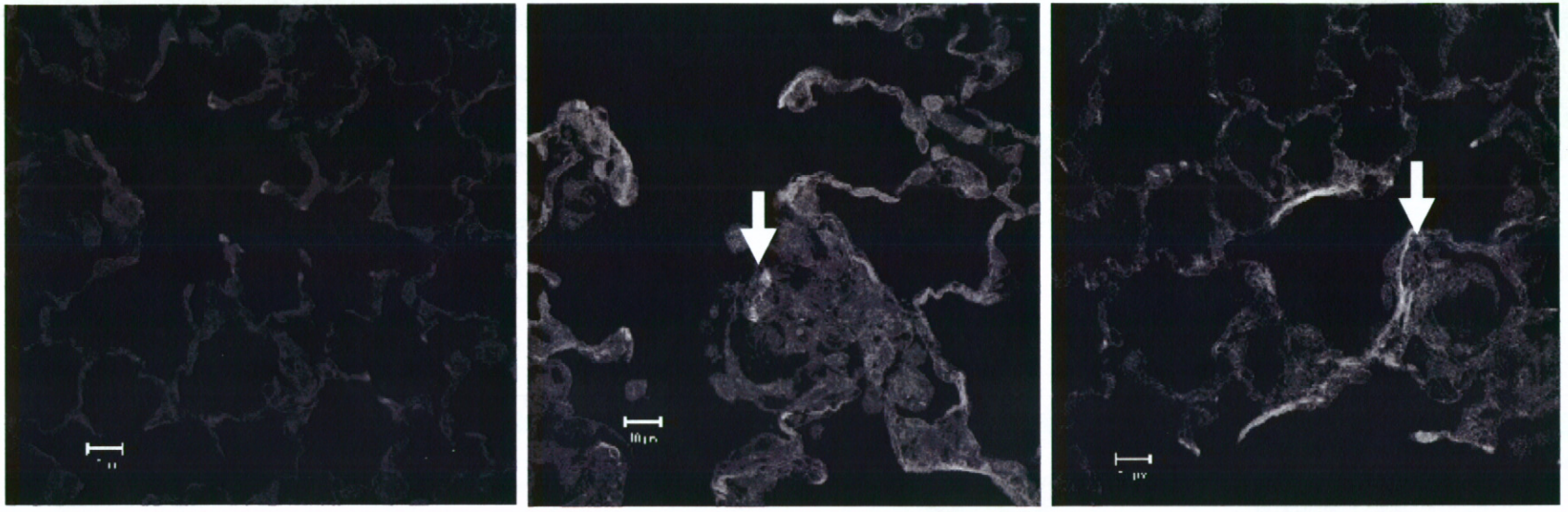
A. CNT types: SWCNT and MWCNT
B. Purity: raw CNT (metal catalysts), purified CNT (treated to remove metals)
C. Structure sizes: agglomerates (poorly dispersed), smaller structures (more dispersed)
D. Methods of pulmonary exposure: pharyngeal aspiration, intratracheal instillation, inhalation (1 – 90 days)

Pulmonary Responses Commonly Reported after CNT Exposure

A. Rapid but transient elevation of BAL markers of pulmonary inflammation and damage (peak at 1-7 days post-exposure then return toward control levels over 3 months post-exposure).
B. Rapid and persistent formation of inflammatory granulomatous lesions at deposition sites of agglomerates.
C. Rapid and persistent interstitial fibrosis associated with the migration of more dispersed structures into the alveolar septa.

MWCNT-Induced Granuloma

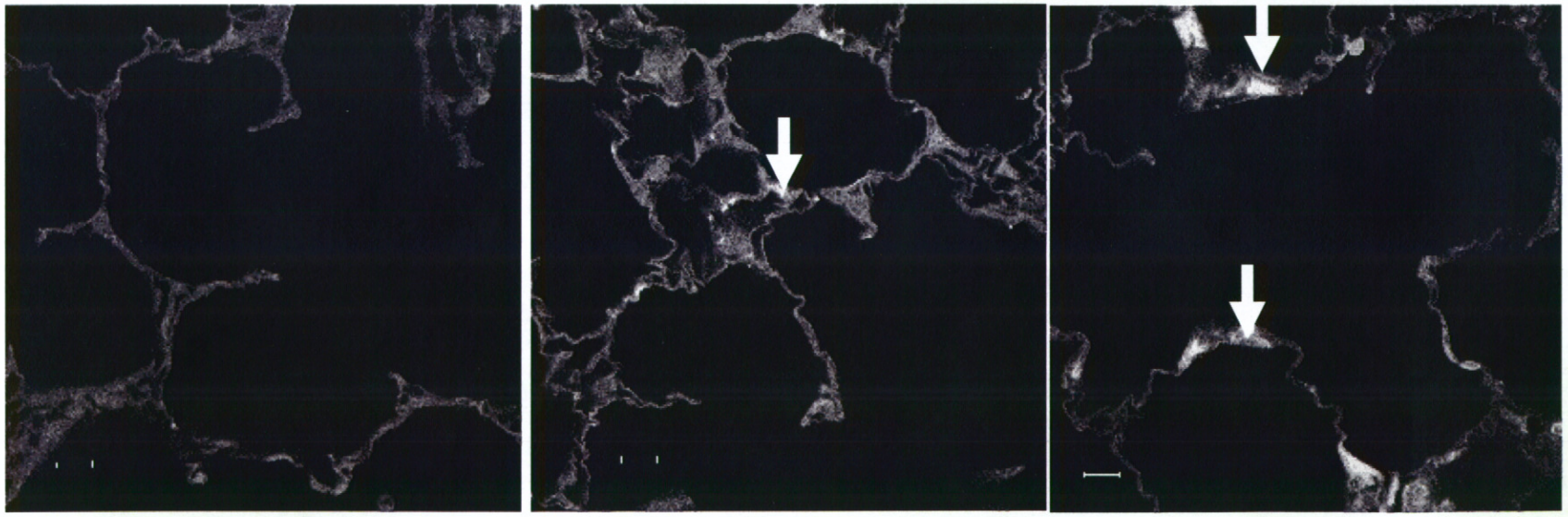




1 day

7 day

30 day



Other Pulmonary Responses Reported after CNT Exposure

A. MWCNT: migration to the subpleural lung tissue and penetration into the intrapleural space.
   1. MWCNT can reach the subpleural tissue 2-6 weeks after inhalation (30 mg/m^3 for 6 hr) (Ryman-Rasmussen et al. Nature Nanotech 4:747-751, 2009)
   2. 12,000 MWCNT in the intrapleural space 56 days post aspiration of 80 µg/mouse (Mercer et al. Particle Fibre Toxicol 7:28, 2010)

MWCNT Penetration of Pleura

Other Pulmonary Responses Reported after CNT Exposure

B. SWCNT: enhanced susceptibility to pulmonary infection
   1. Pretreatment of mice with 40 μg SWCNT for 3 days
   2. 10 day after aspiration of Listeria
   3. 5 fold increase in bacterial CFU from lung tissue (Shvedova et al. Am J Respir Cell Mol Biol. 38: 579-590, 2008)

Other Issues of Pulmonary Concern with CNT

A. Disruption of mitosis
   1. Bronchial epithelial cells *in vitro*
      a. SWCNT – multipolar
      b. MWCNT – monopolar

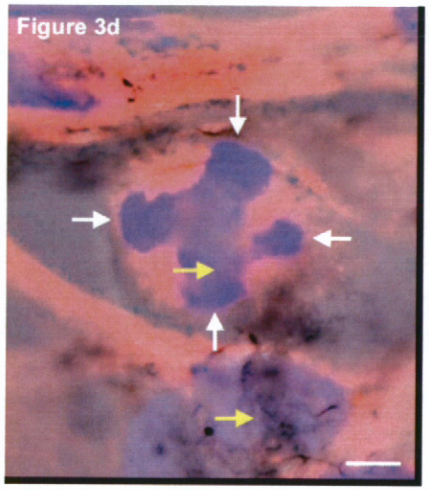
      (Sargent et al. Environ Mol Mutagen 50: 708-717, 2009)

B. Cell transformation
   1. Bronchial epithelial cells exposed to CNT (low dose 0.02μg/cm^2; long term (25 weeks) *in vitro* exposure)
   2. Increased –cell proliferation, invasive potential, growth in soft agar
   3. SWCNT more potent than MWCNT (Stueckle et al. The Toxicologist, 2011)

In Vitro Genotoxicity

II. Effect of SWCNT on BEAS-2B cells.

E. SWCNT alter the number of spindle poles



Red= spindle tubulin, blue = DNA, black = SWCNT

(Sargent et al. Environ. Mol. Mutagen., 2009)

Other Issues of Pulmonary Concern with CNT

C. Mesothelioma

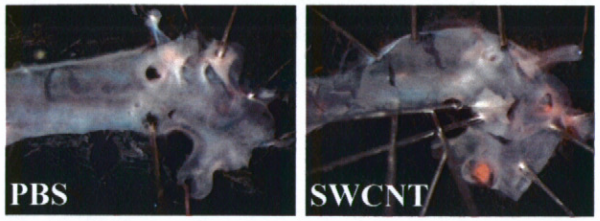
1. Abdominal injection of a high dose of MWCNT induced mesothelioma (Takagi et al, J Toxicol Sci. 33: 105-116, 2008).
2. Long MWCNT more potent in causing granulomatous lesions on the diaphragm (Poland et al. Nature Nanotech 3:423-428, 2008)
3. Intrascrotal injection of MWCNT caused abdominal mesothelioma (Sakamoto et al, J Toxicol Sci 35:65-76, 2009).

Systemic Issues with CNT

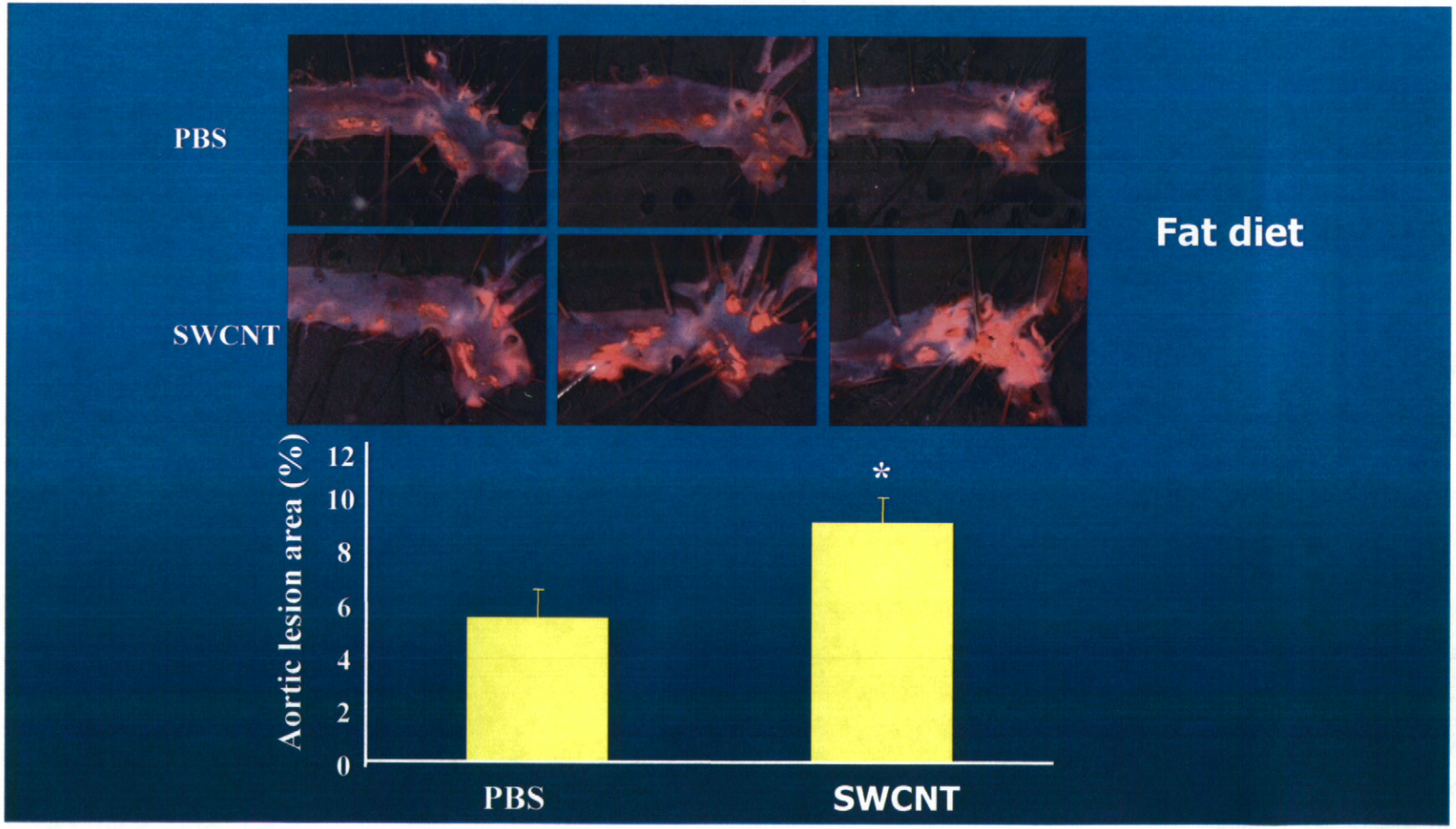
A. Cardiovascular effects of pulmonary exposure
   1. Multiple aspirations (20 μg/mouse, 4x) to SWCNT in Apo E -/- mice increased aortic plaques (Li et al. Environ Health Perspect 115: 77-82, 2007).
   2. Inhalation (5 hr at 26 mg/m^3 to give a 22 μg lung burden) MWCNT in rats. Blocked dilation of coronary arterioles in response to acetylcholine 24 hr post-exposure (Stapleton et al, The Toxicologist, 2011)

ApoE-/- mice - multiple exposure

En face aorta Sudan IV staining



Regular diet



Systemic Issues with CNT

B. CNS effects of pulmonary exposure

1. Aspiration of MWCNT (80 μg) in mice; 24 hr post-exposure
2. Increased mRNA for inflammatory chemokines and cytokines as well as selectins (markers of blood/brain barrier damage) in the olfactory bulb, frontal cortex, midbrain and hipocampus. (Sriram et al. The Toxicologist 108: A2197, 2009)

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)

Risk Analysis using Available Pulmonary Toxicology Data

C. Using granulomatous inflammation or interstitial fibrosis calculate the animal model lung burden giving 10% risk (benchmark dose).

D. From the benchmark dose calculate the workplace airborne concentration which would result in this lung burden in a working lifetime (5 d/w, 50 w/yr, 45 yrs)

Calculation of Benchmark Workplace Level for Human Exposure

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

Study	Exposure	Species	Benchmark Exposure Level ($\mu g/m^3$)
Muller et al (2005)	IT	Rat	18
Porter et al (2010)	aspiration	Mouse	0.6
Ellinger – Zieglbauer & Pauluhn (2009)	Inhalation	Rat	3.8
Pauluhn (2010)	Inhalation	Rat	0.8
Ma-Hock et al (2009)	Inhalation	Rat	0.5

Summary

A. Studies with SWCNT or MWCNT

1. Mice or rats
2. Raw or pure
3. Agglomerated or more disperse
4. Bolus or inhalation exposure

Give qualitatively similar responses: rapid and persistent inflammatory granulomas and/or interstitial fibrosis

Summary

B. Other responses requiring further research:

1. Lung cancer
2. Mesothelioma
3. Cardiac dysfunction
4. CNS changes