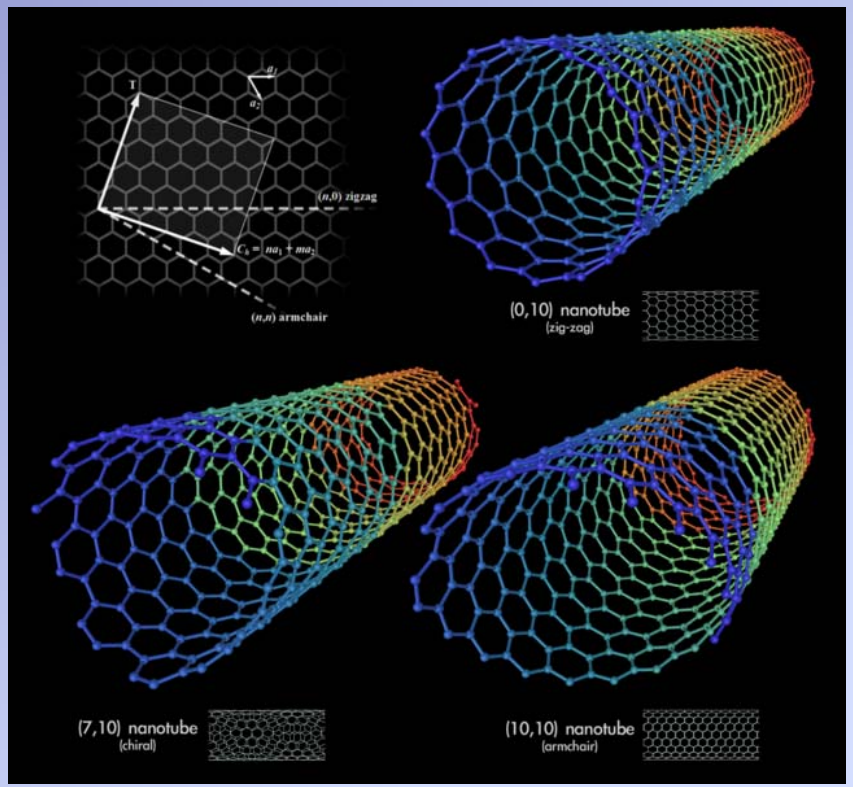
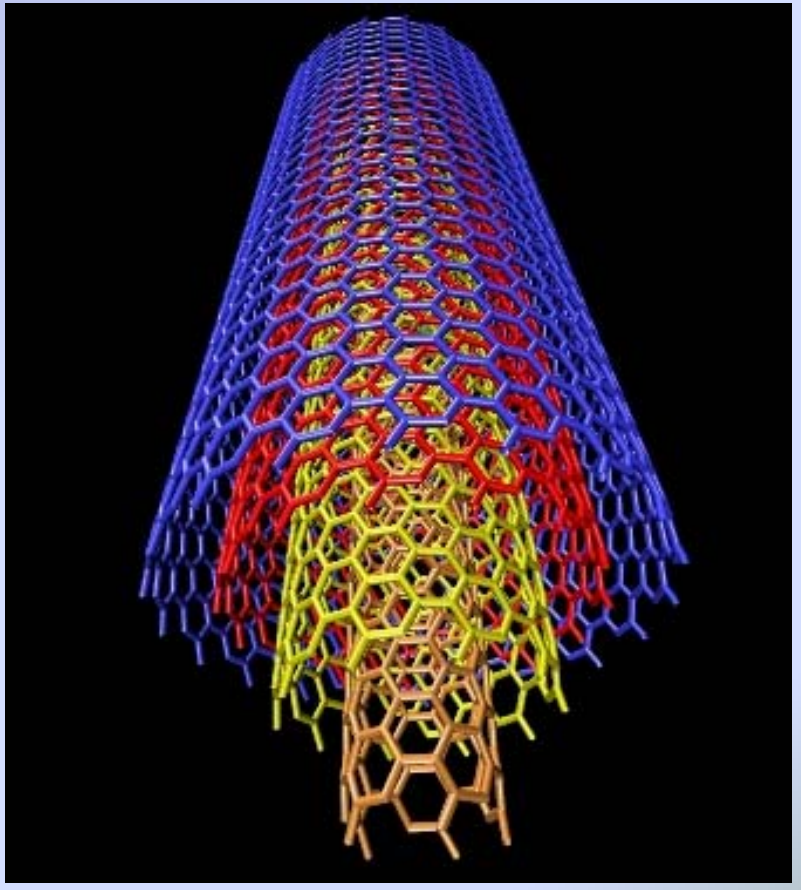
Part 2: Focus on Carbon Nanotubes

Carbon Nanotubes (CNTs)

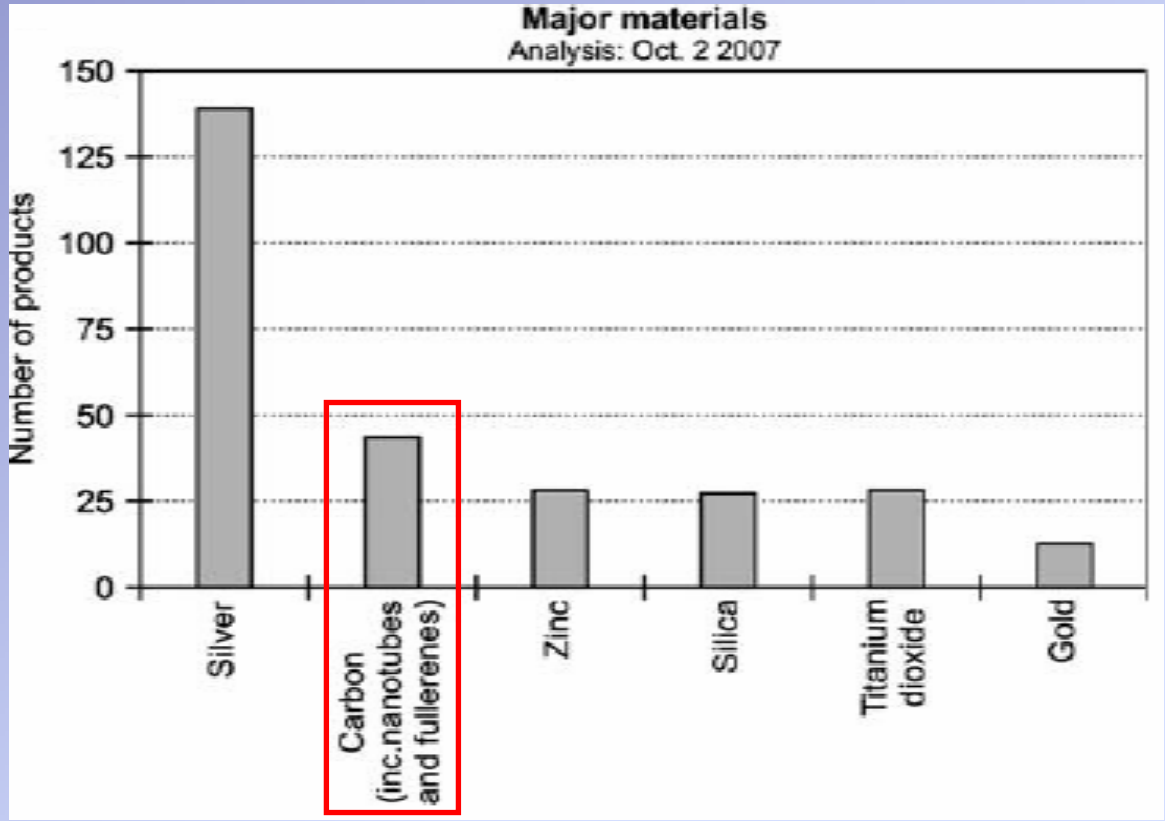




Single-walled CNT

Multi-walled CNT

Nanomaterials in Consumer Products



From Hansen et al., 2008

Wilson Center Project on Emerging Nanotechnologies (PEN):

580 consumer products

4 therapeutic products (FDA-approved)

Are Carbon Nanotubes Dangerous to Human Health?

Hazardous fiber characteristics:

Long, thin, biopersistent

Hazard x Exposure = Risk

Lessons from In Vitro Assessments of CNT Toxicology

- Nonpurified CNTs induced higher oxidant release, lipid peroxidation, and GSH depletion in macrophages than purified CNTs with 100x lower Fe content (Kagan et al., 2006)
  - reversible with catalase
- "Unrefined" CNTs induced lipid peroxidation and antioxidant enzyme depletion in keratinocytes (Shvedova et al., 2003)
- CNTs induce cytotoxicity and impairment of phagocytosis in primary macrophages (Jia et al., 2005)

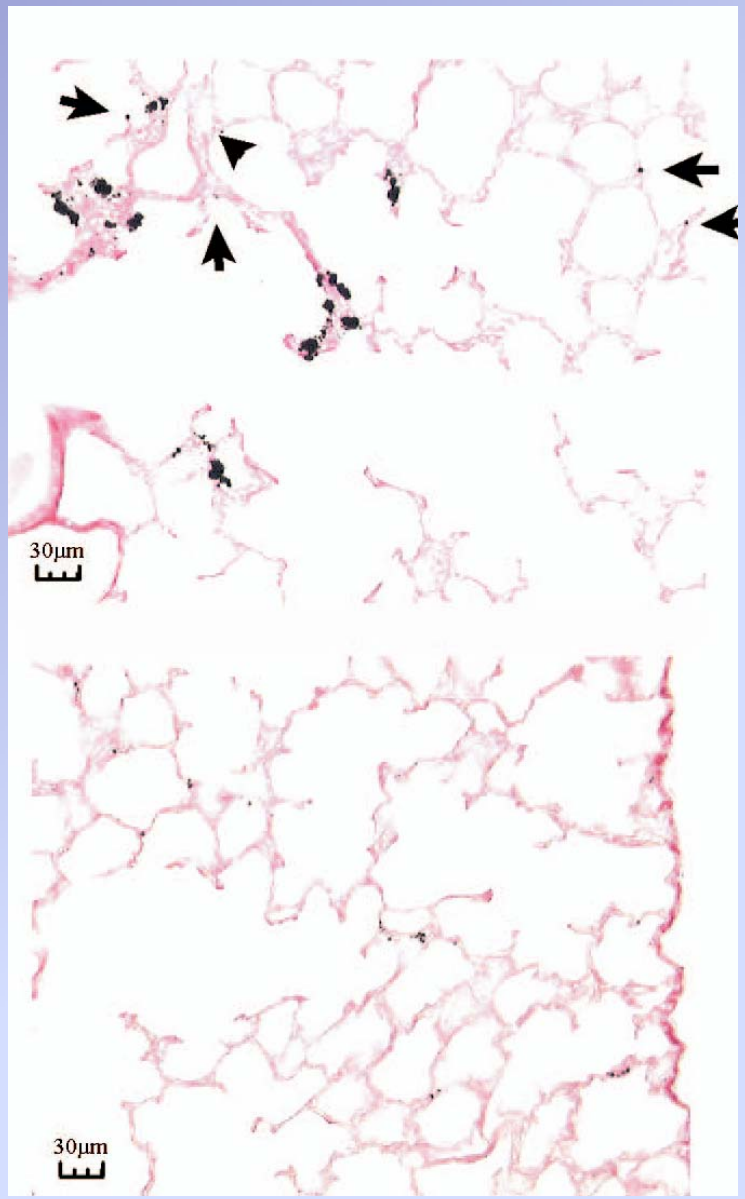
Caveats for *In Vitro* Studies

1. Cell type selection
2. Dose … realistic in terms of target cell exposure conditions?
3. Cytotoxicity assay results need careful interpretation (best to use combination of assays):
   - Assays involving the formation of insoluble formazan salts produce false-positive results (*Casey et al., 2007; Worle-Knirsch et al., 2007*)

Effects of CNTs in the Respiratory Tract:
Studies using Aspiration or Instillation Exposures

- Early studies in mice and rats reported deaths and granuloma formation in lung (Lam et al., 2004; Warheit et al., 2004).
- Lower dose studies showed dose-related increases in granulomas, interstitial fibrosis, inflammation, and markers of oxidative stress (GSH depletion, 4-HNE, inflammatory mediators) (Shvedova et al., 2005).
  - Granulomas with aggregated CNTs, diffuse fibrotic changes where CNTs not visible
- SW-CNT pre-exposure reduced clearance of bacteria and decreased phagocytosis (Shvedova et al., 2008)

Effects of CNTs in the Respiratory Tract



Undispersed

Dispersed

from Mercer et al., 2008

Effects of CNTs in the Respiratory Tract:
Studies using Inhalation Exposures

- Inhalation of MW-CNTs leads to milder pathology as compared to instilled material (Li et al., 2007).
- CNTs delivered via inhalation caused 2-4 times higher lung inflammation (cellular influx, cytokines) and fibrotic changes than in mice exposed via oropharyngeal aspiration (Shvedova et al., in press)

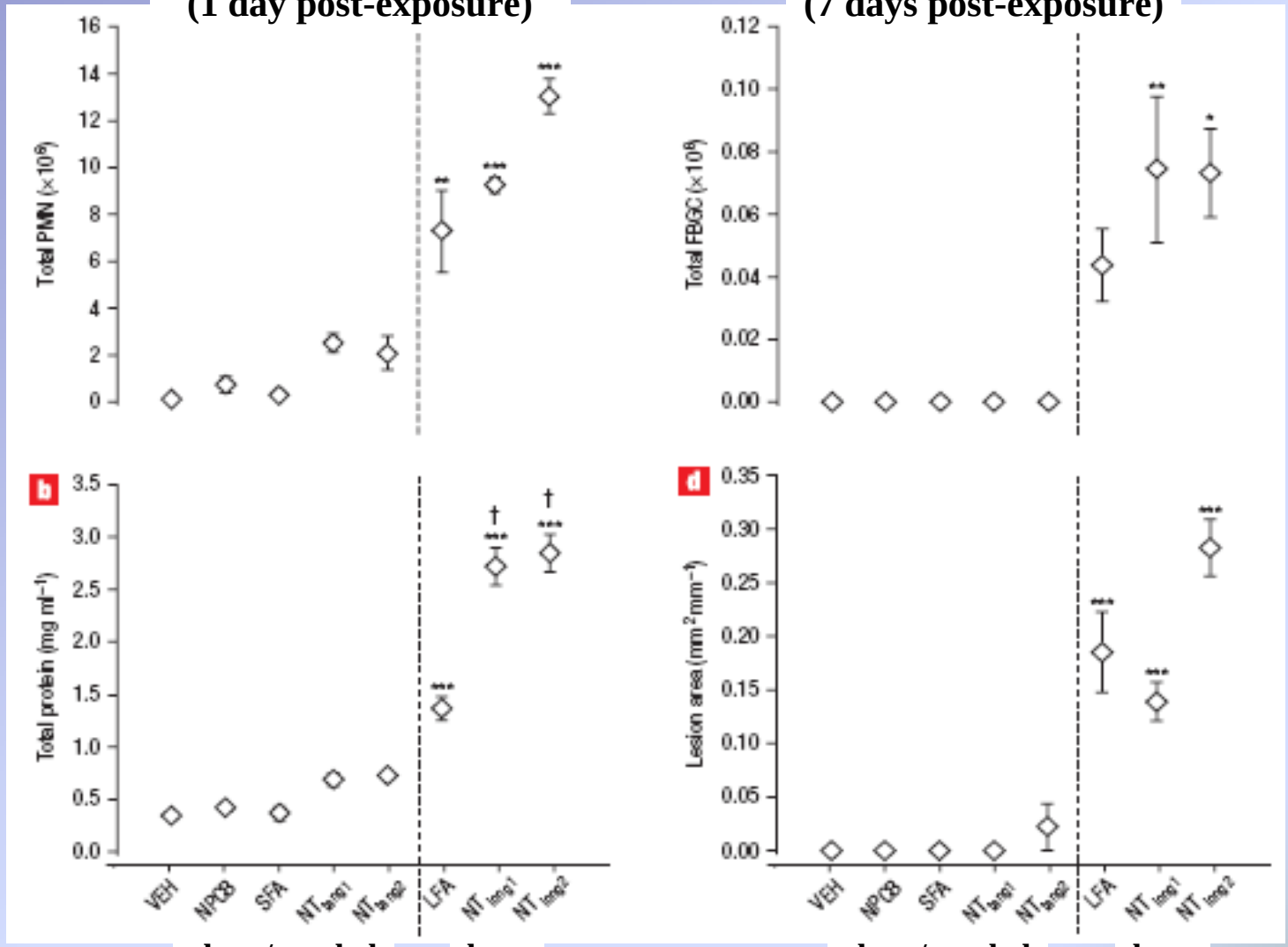
Do MW-CNTs Behave Like Asbestos in *In Vivo* Studies?

1. Poland et al., 2008 (*Nature Nanotechnol.*)
   - Intraperitoneal injections (50 μg) in mice;
   - Compared 4 CNT preparations to carbon black and short and long amosite fibers.
2. Takagi et al., 2008 (*J. Toxicol. Sci.*)
   - Intraperitoneal injections (3 mg) in $p53^{+/-}$ mice;
   - Compared to crocidolite fibers and fullerene.

Do MW-CNTs Behave Like Asbestos?

Inflammatory Response (1 day post-exposure)

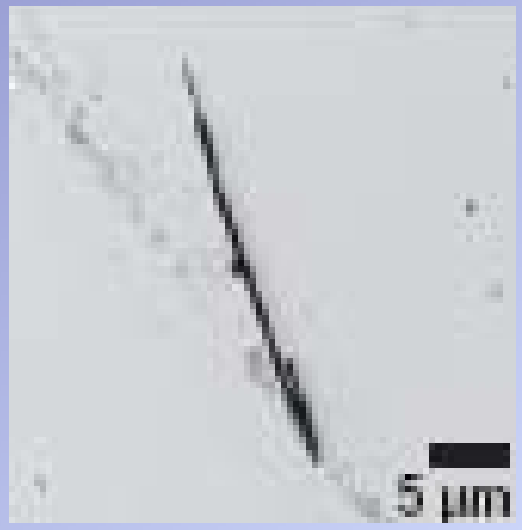
Granuloma Response (7 days post-exposure)



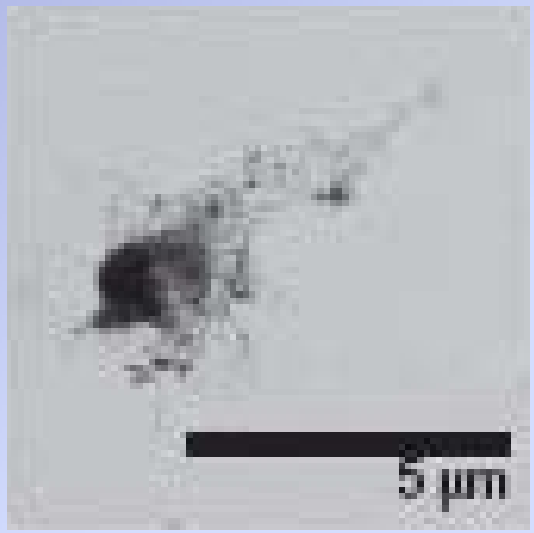
short/tangled **long** **short/tangled** **long**

from Poland et al., 2008

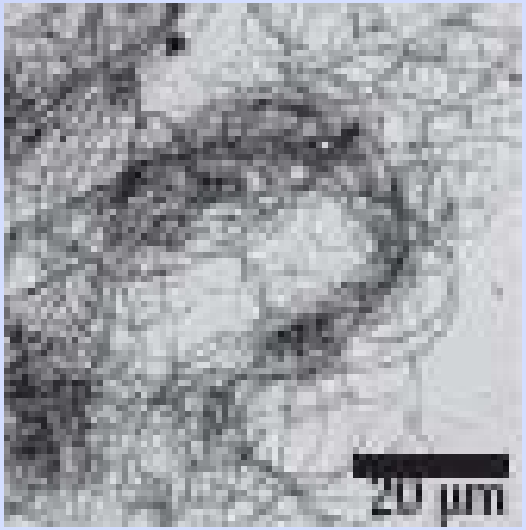
Do MW-CNTs Behave Like Asbestos?



Long-fiber amosite



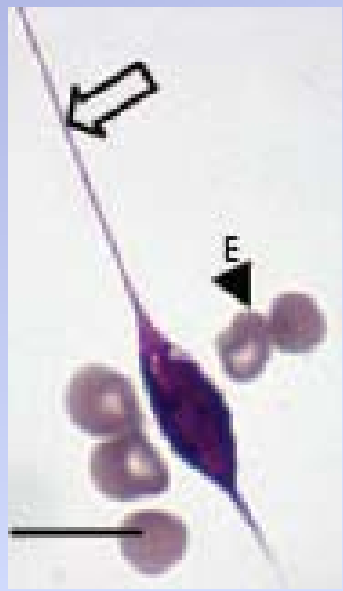
Tangled CNTs

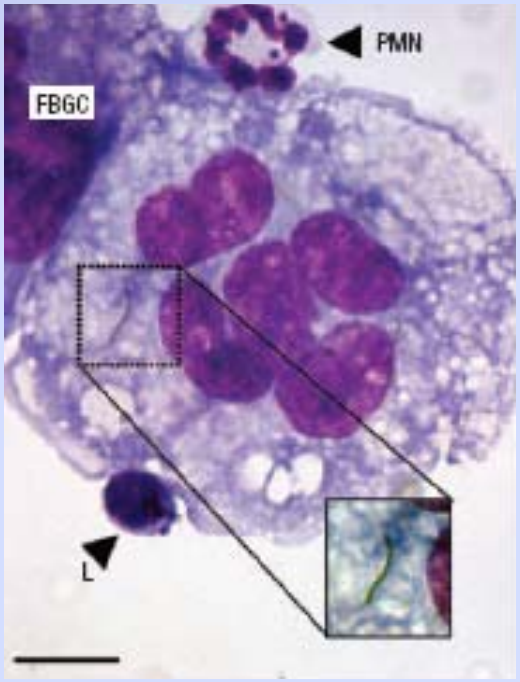


Long CNTs

from Poland et al., 2008

Do MW-CNTs Behave Like Asbestos?



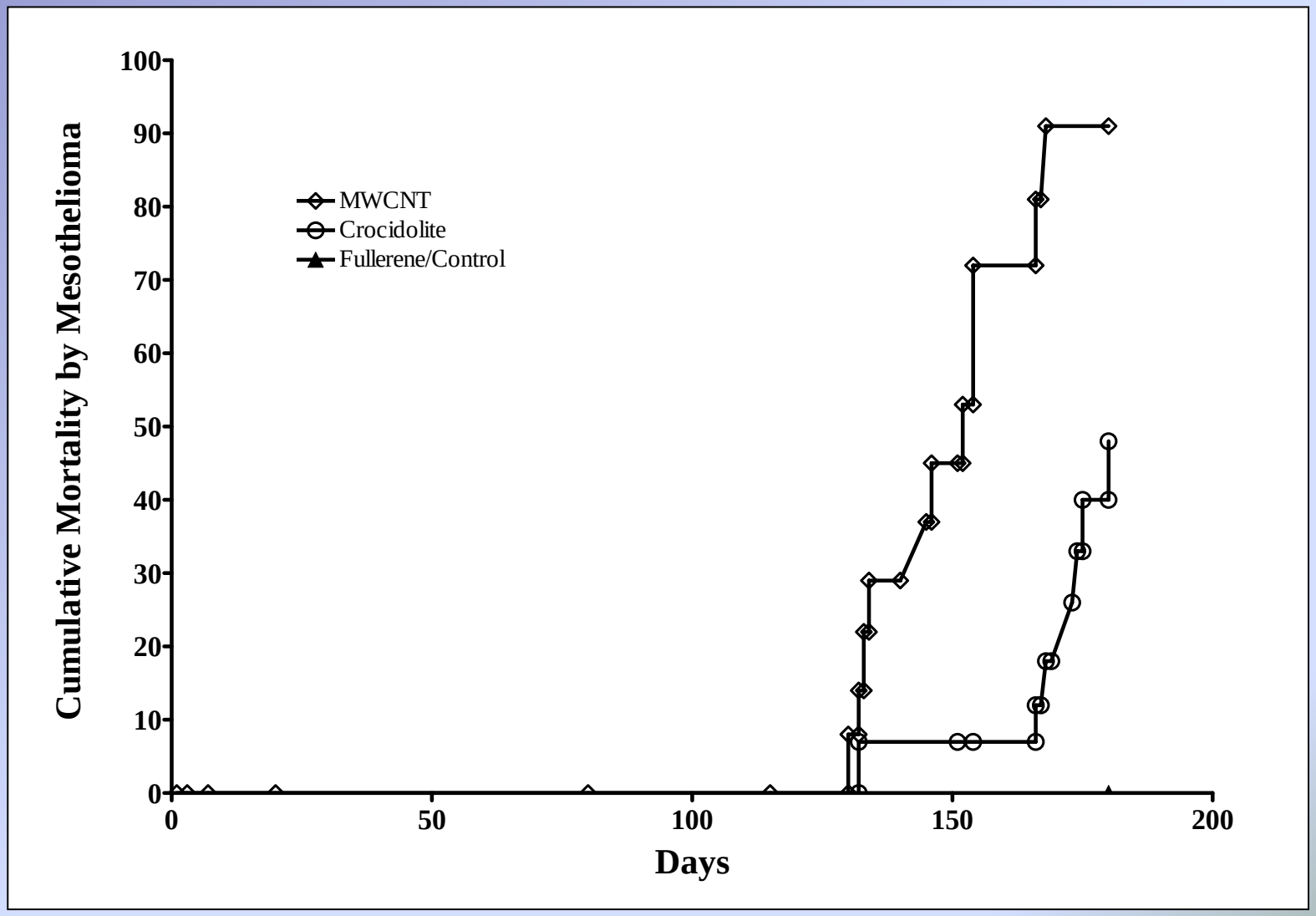


Long-fiber amosite

Long CNTs

from Poland et al., 2008

Do MW-CNTs Behave Like Asbestos in p53$^{+/-}$ Mice?



Adapted from Takagi et al., 2008

Important Considerations

1. Right target cell (for mesothelioma)
2. Comparison to amphiboles and other carbon-based materials

1. Huge doses
2. Dose metric = mass
   - numbers of fibers of different lengths not considered
3. Route of exposure, dose rate

Point of commonality: MW-CNTs > amphibole

Workplace CNT Exposures

- Maynard et al. (2004) measured airborne levels of **respirable** particles in a small university laboratory
  - peak mass concentration, ~53 $\mu g/m^3$
- Han et al. (2008) measured airborne levels of **total** particulate in a research laboratory
  - undetectable to ~400 $\mu g/m^3$
  - control measures reduced dustiest operations to undetectable levels

Further Considerations

1. Interpretation of results from *in vitro* assays:
   - Interference of CNTs with insoluble formazan products (Casey et al., 2007; Wörle-Knirsch et al., 2007)
2. For *in vivo* studies, don’t discount smaller fibrous particles.
3. Intraperitoneal injection model is not universally accepted.