Epidemiologic studies of U.S. workers handling carbon nanotubes: the interface between exposure and health

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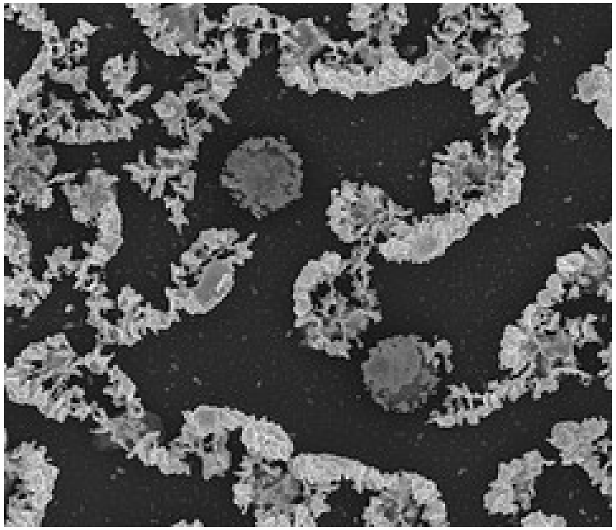
Health concerns about nanomaterials

- Special properties of nanomaterials may enhance toxic potential
  - Small size
  - Large surface area per unit mass
  - High aspect ratio
  - Different surface charge
- Long, thin shape may confer asbestos-like properties
  - Some CNT & carbon nanofibers (CNF)
  - Metal nanowires or nanocellulose



Silver-Oxide Coating on Surface of Silicon Wafer, image courtesy of Samuel Peppernick

Health concerns about nanomaterials

Toxicological & environmental studies of nanoparticles suggest possible:

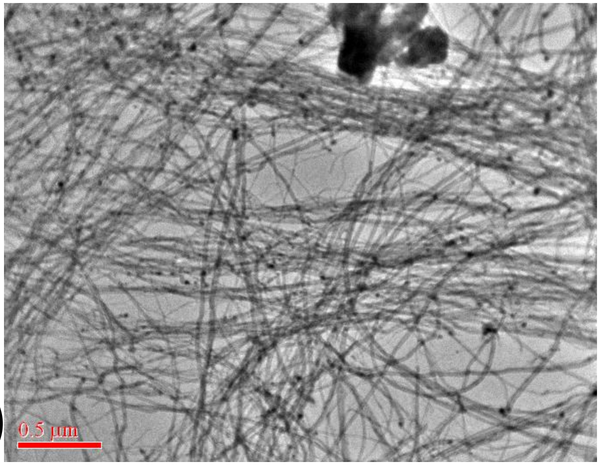
--Pulmonary effects (CNT & CNF)

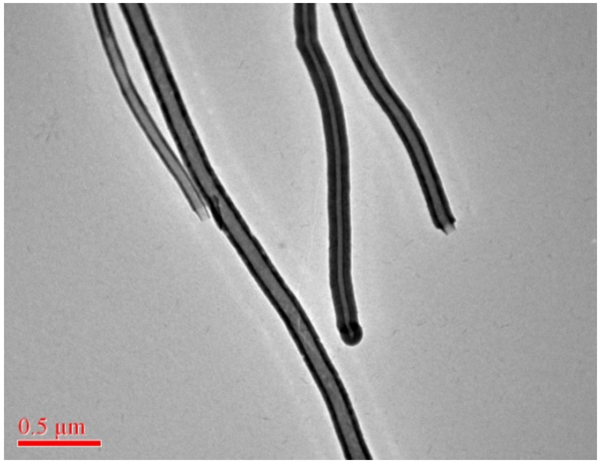
- Pulmonary fibrosis
- Penetration of pleura
- Mitotic disruption (mutagenesis)
- Lung tumor promotion

--Cardiovascular effects (air pollution epidemiology)

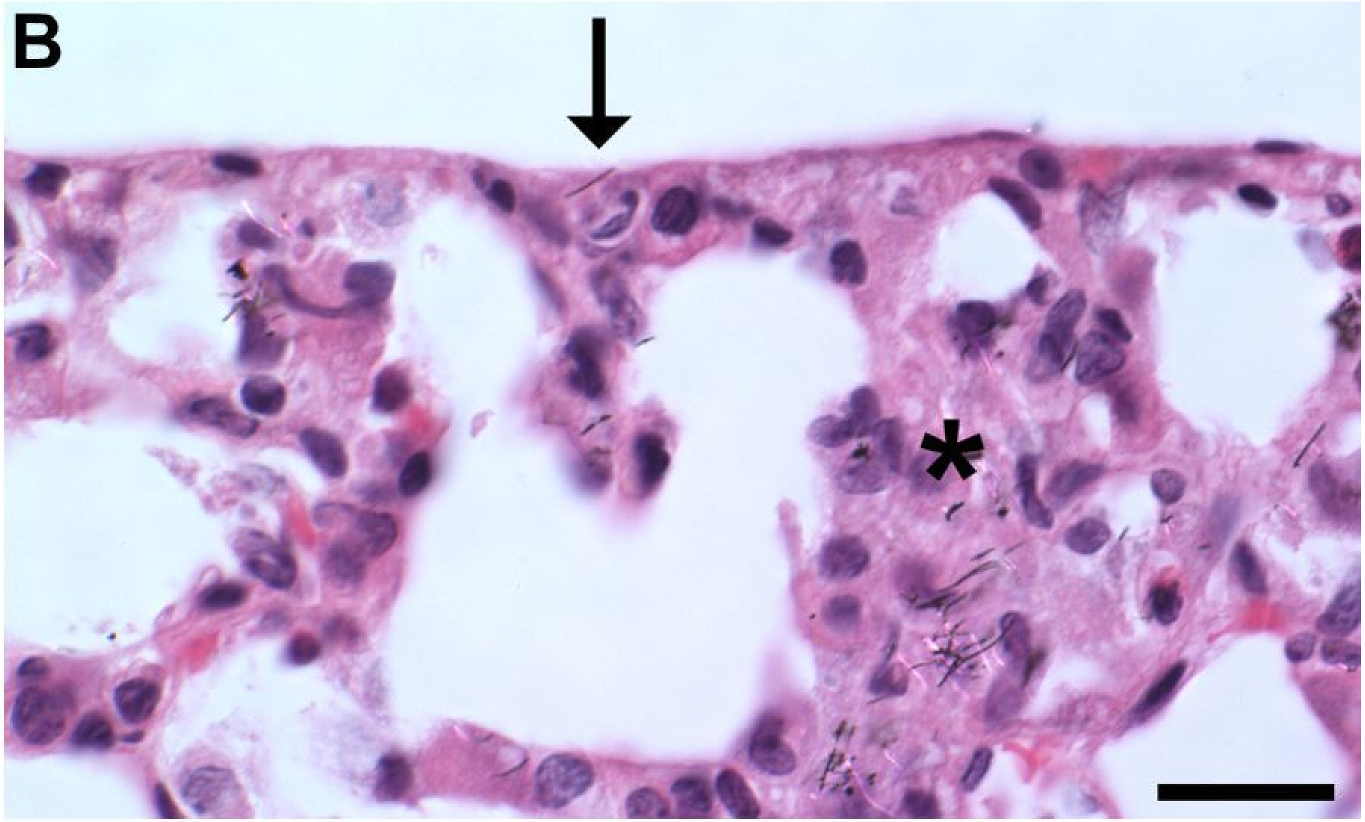
- Decreased heart rate variability
- Arterial vasoconstriction
- Increased blood pressure
- Higher plasma viscosity

--Initiation of inflammatory cascade





Multi-walled CNT Reaching Pleural Surface



7 days post-exposure; 40 µg aspiration in mice; *indicates site of persistent fibrosis; [Porter et al. Toxicology 269 (2010) 136–147]

Challenges in studying engineered nanomaterial workers

Small workforce sizes

- Findings in U.S. workforce handling engineered carbonaceous nanomaterials (ECN) (Schubauer-Berigan et al. 2011; 2013):
  - N = ~650 in 2009
  - Growing at 15% annually
  - 75% of workforce handling CNT & CNF, which is growing at 22% annually
- Industry characterized by a high degree of automation, even for large-scale production

Challenging materials

- Typical CNT diameters: 1-50 nm, lengths of 1-100 µm
- Typical daily quantities handled in grams
- Air concentrations associated with significant health effect (e.g., 1 µg/m^3) for CNT as elemental carbon (EC)

Short latency: materials only recently commercialized

How do we prioritize engineered nanomaterials for occupational epidemiologic study?

1. Degree of potential hazard
   - Results of toxicological studies, including mechanistic information
   - Analogy from other materials (air pollution, asbestos)
2. Potential for exposure
   - Number of workers
   - Quantity of materials used
   - Results of exposure assessments

NIOSH approach: Phased industrywide studies

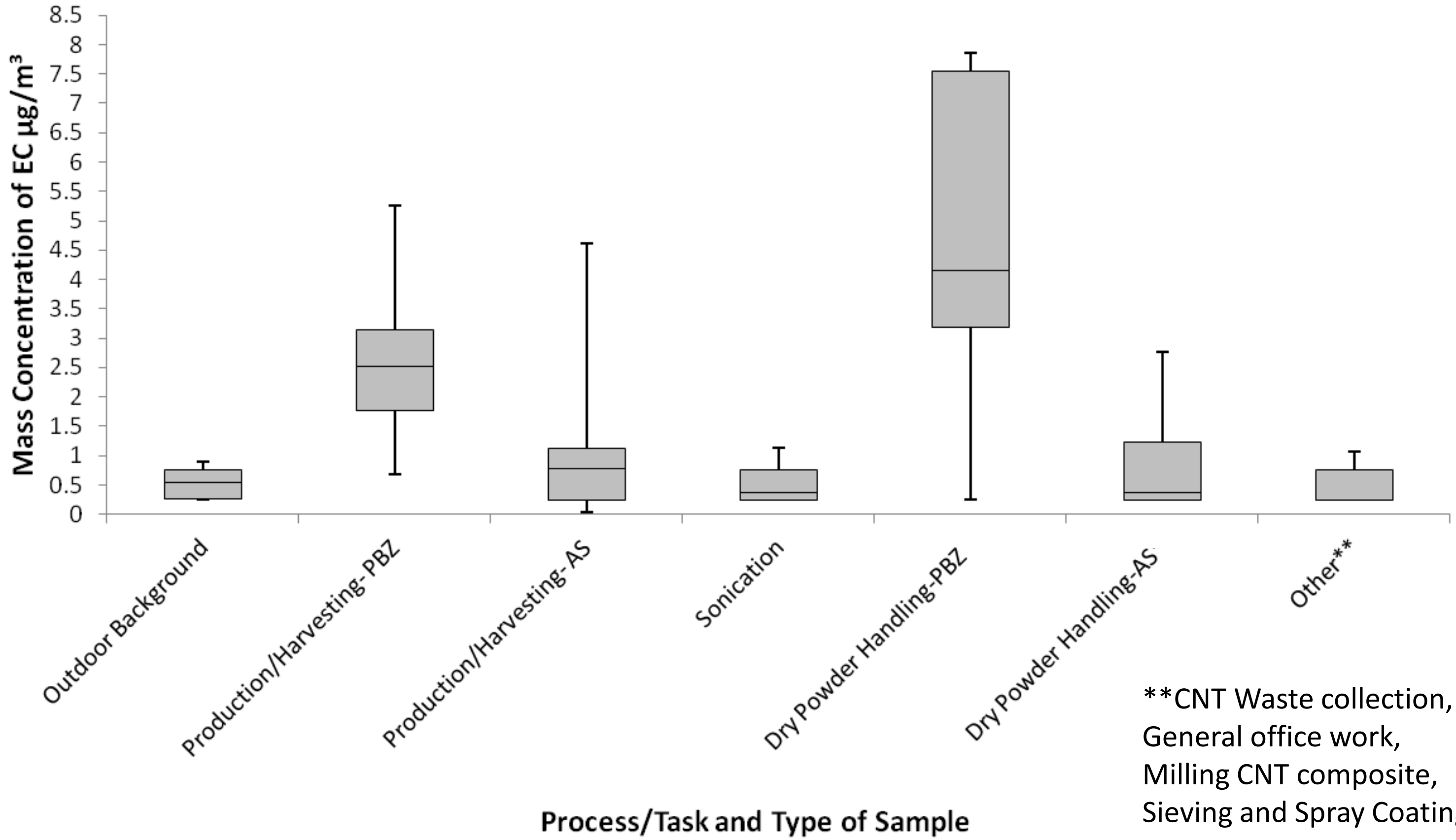
Phase I: Collected information to determine feasibility of industrywide studies of engineered carbonaceous nanomaterial (ECN) workforce • Estimated U.S. workforce size and growth for different types of ECN in companies larger than R&D
Phase II: Conducted industrywide exposure assessment for CNT & CNF, the most widely used ECN • Optimized methods to measure CNT & CNF exposure in workplaces
Phase III: Conduct epidemiologic studies • Evaluating markers of early biological effects in relation to metrics of exposure and develop prospective cohort

Phase II: Industrywide exposure assessment study

- Conducted 2010-2012 (Dahm et al. 2012, 2013, 2015)
- Objectives:
  - Develop methods to measure exposures to CNTs and CNFs at biologically relevant levels using key exposure metrics.
    - Filter based: EC & size-specific fiber concentrations
    - Direct reading instruments (DRI): particle number, active surface area
  - Characterize full-shift and task-specific exposures in a representative sample of primary and secondary manufacturers
- Findings: both inhalable and respirable EC mass and TEM structure count concentrations should be used in epidemiologic study

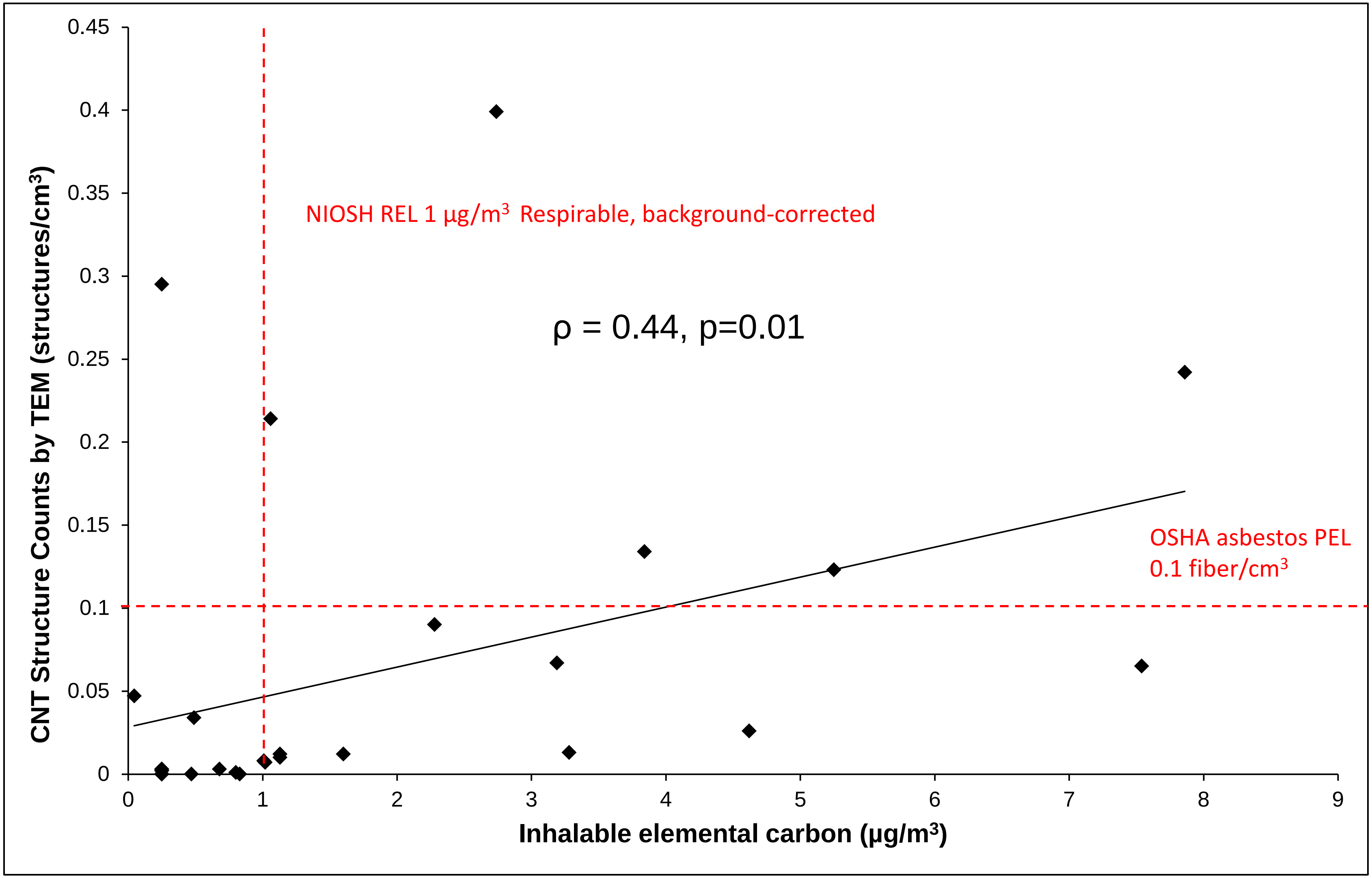
Variation in elemental carbon exposure by task

(Dahm et al. 2012)



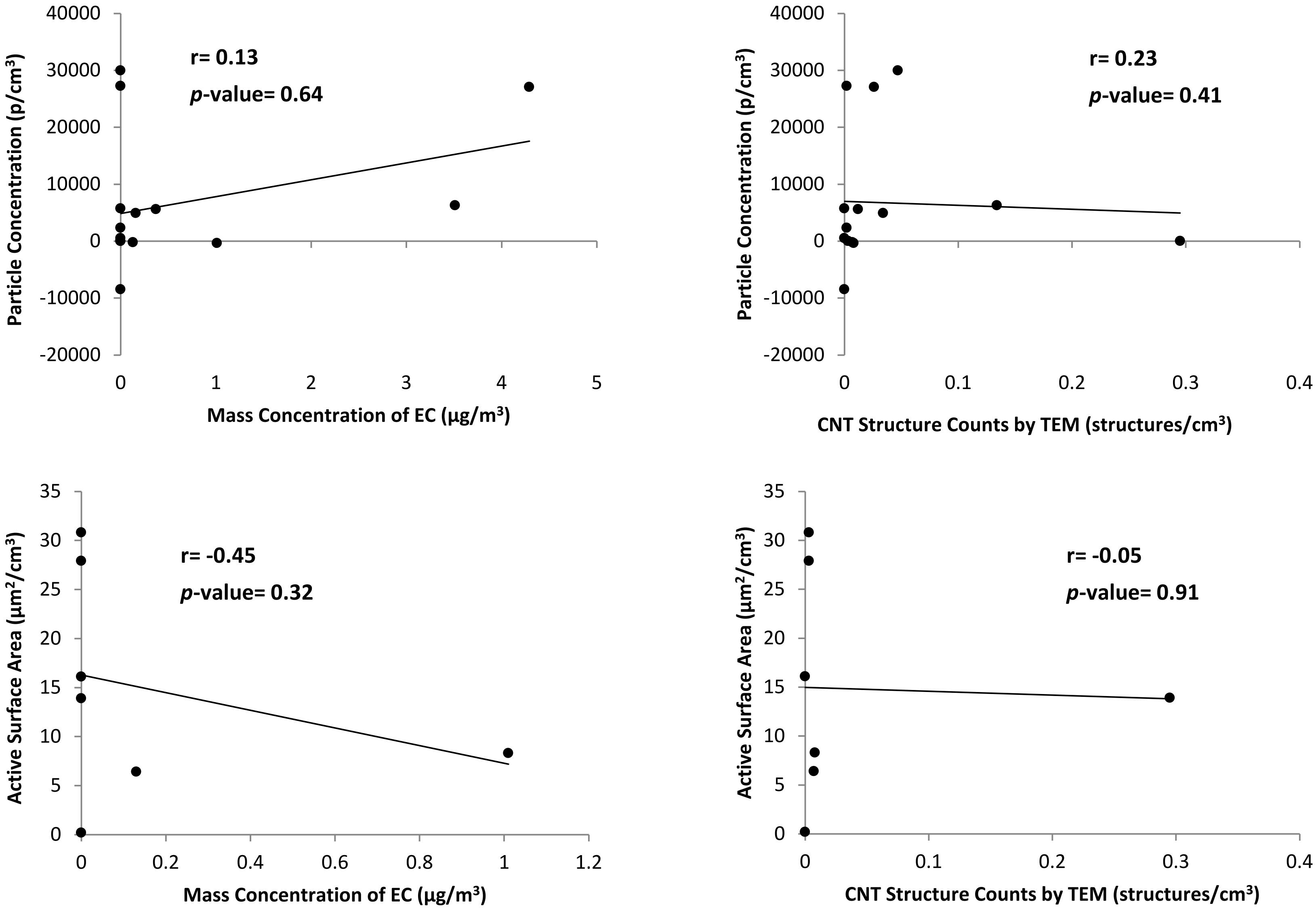
**CNT Waste collection, General office work, Milling CNT composite, Sieving and Spray Coating

Correlation of EC vs. TEM filter-based



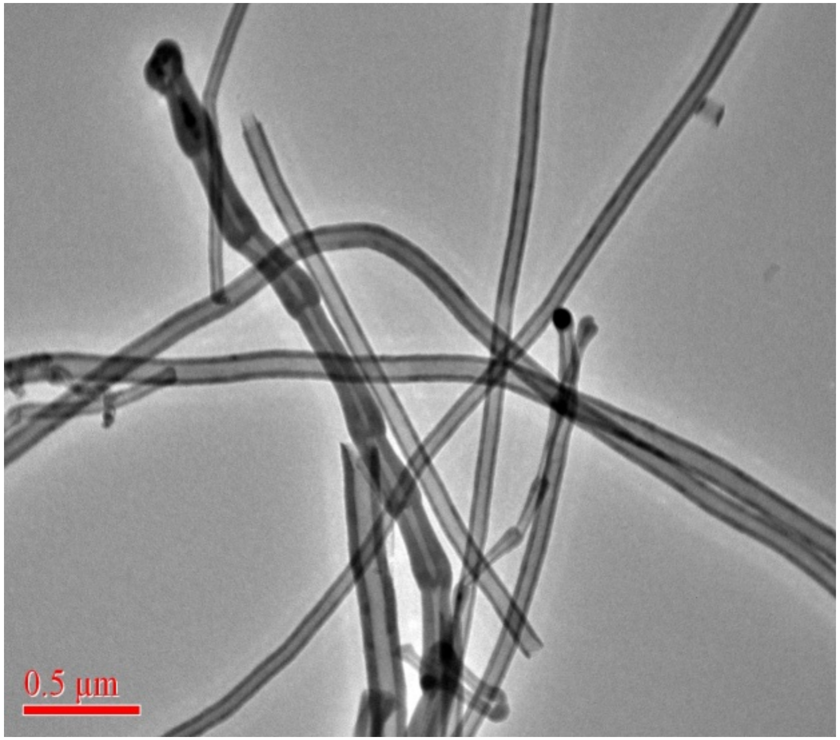
Adapted from Dahm et al. (2012)

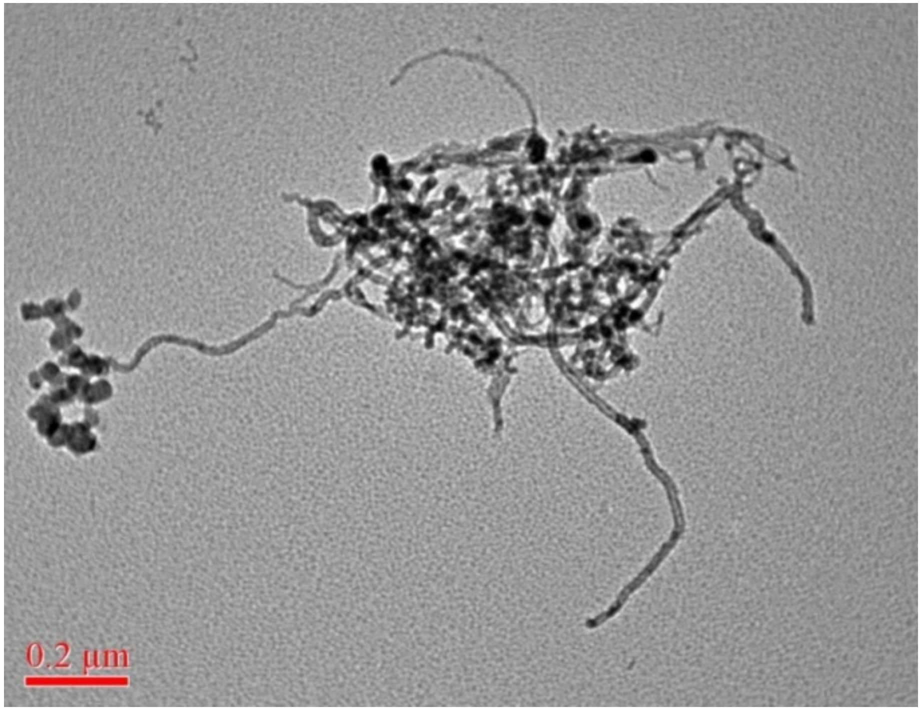
Correlation of background-corrected DRI with filter-based samples (Dahm et al. 2013)



Exposure Assessment Challenges

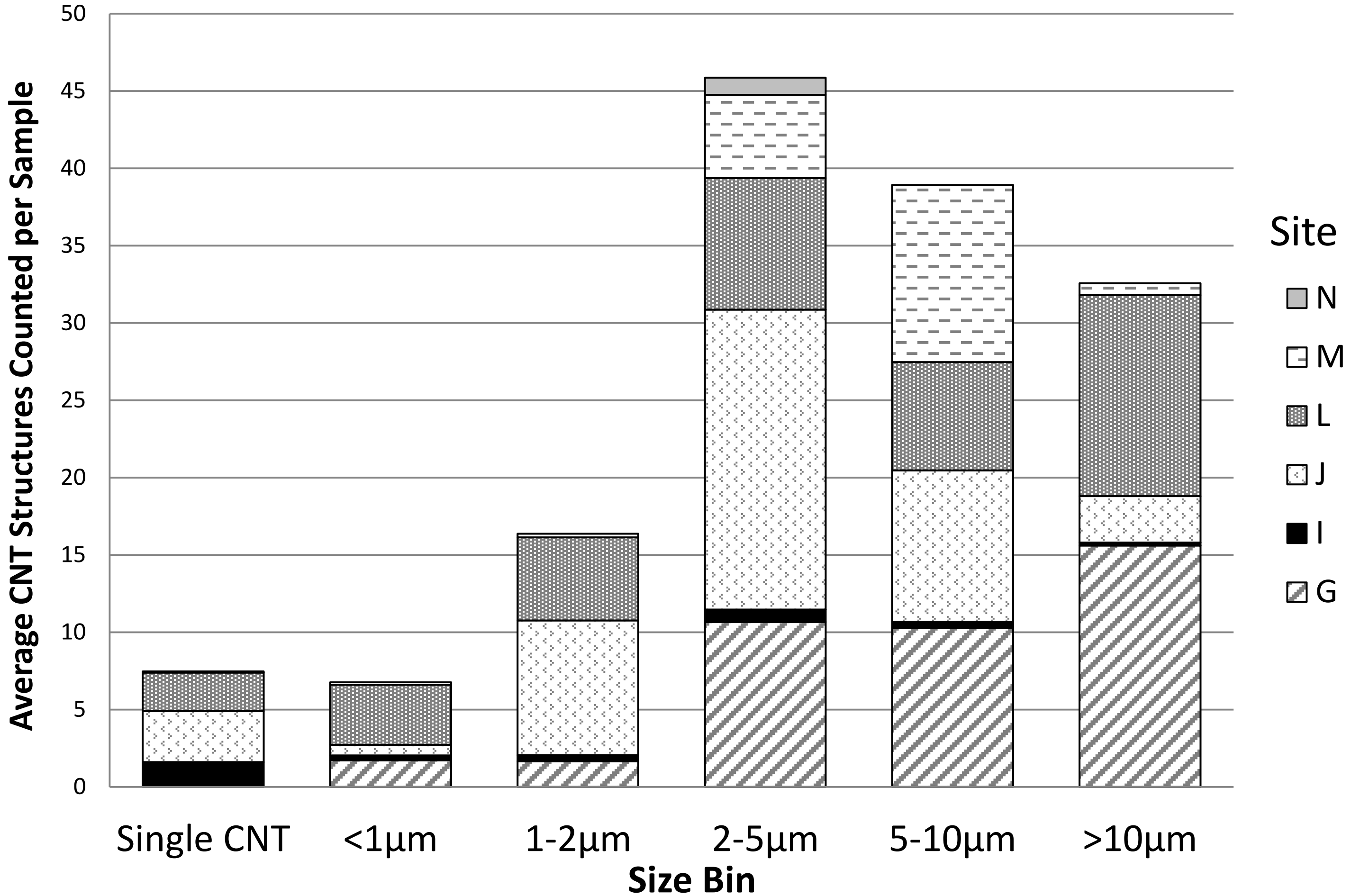
- Do these two structures have the same potential for toxicity?





Images from personal breathing zone samples from CNT manufacturing (Dahm et al. 2012)

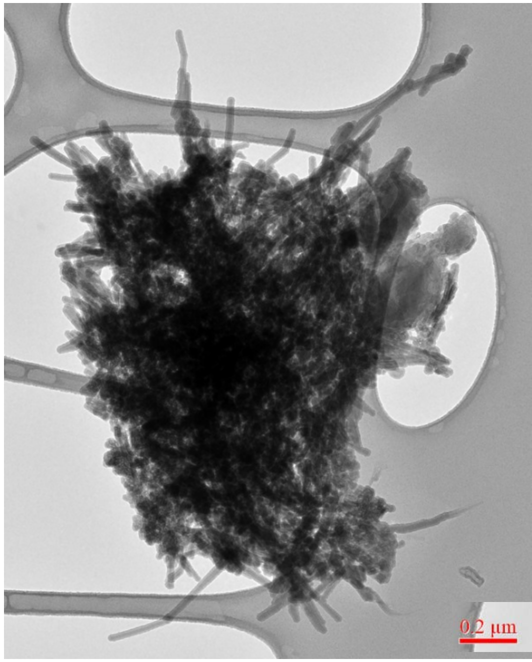
Examples of CNT Structures by Size

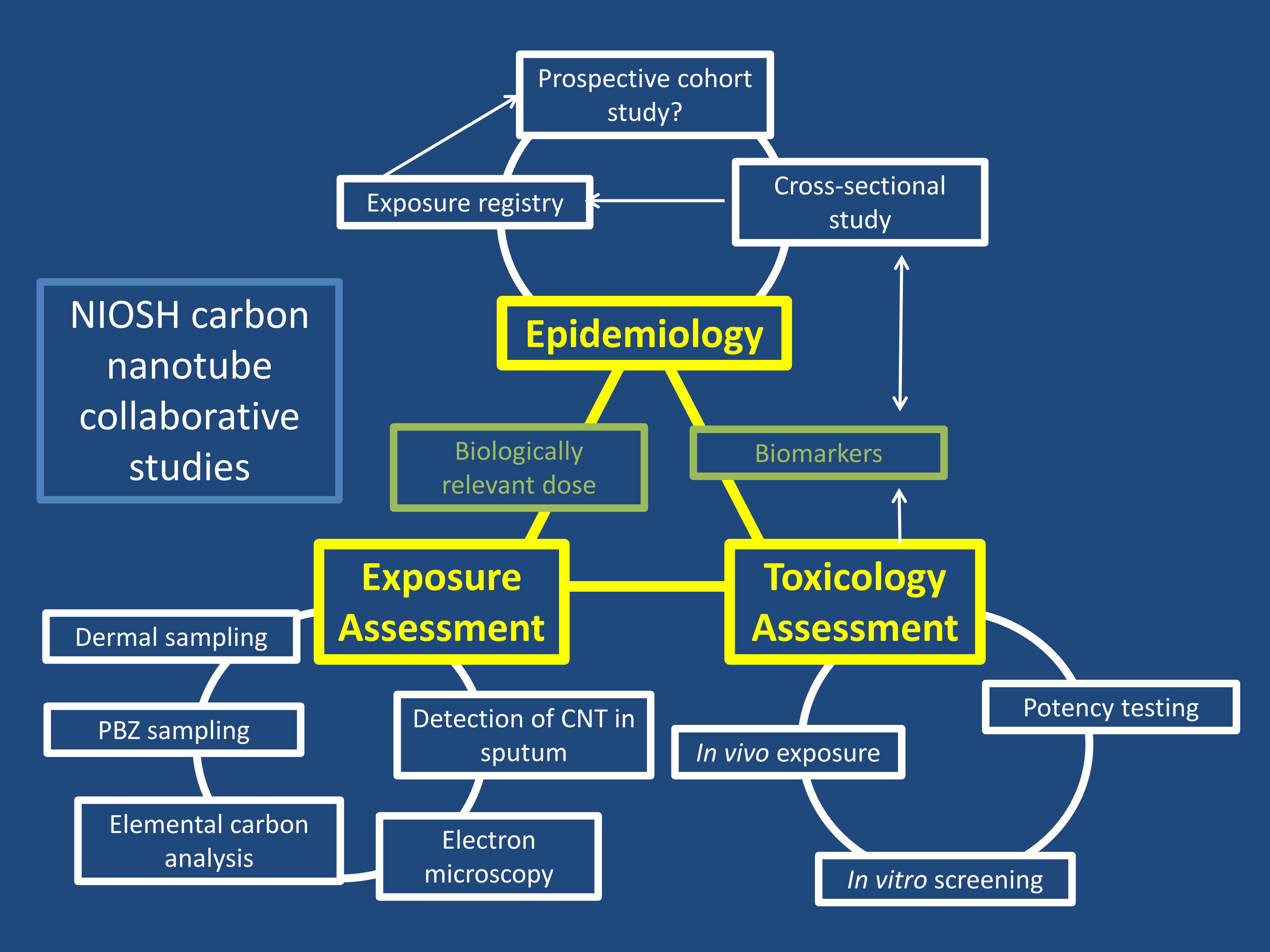


(Dahm et al. 2015)

Possible exposure determinants

- Synthesis method, for primary manufacturers
- Type and toxicity of raw materials
- Nominal aspect ratio of CNT or CNF
- Form of CNT and CNF used—dry powder, liquid
- Coatings
- Type of processes and tasks performed by worker
- Use & adequacy of personal protective equipment
- Length of shift
  - Time spent working directly with CNT or CNF
  - Time spent potentially indirectly exposed to CNT or CNF
- High-concern activities: Harvesting, dry powder handling, cleaning operations and waste disposal




NIOSH carbon nanotube collaborative studies
Prospective cohort study?
Exposure registry
Cross-sectional study
Epidemiology
Biologically relevant dose
Biomarkers
Exposure Assessment
Toxicology Assessment
Dermal sampling
PBZ sampling
Elemental carbon analysis
Detection of CNT in sputum
Electron microscopy
In vivo exposure
Potency testing
In vitro screening

Phase III: Epidemiologic studies

A. Cross-sectional study (in progress)

- Carbon nanotube and nanofiber-exposed workers
- Measures of early health effects
- Measures of best exposure metrics:
  - Elemental carbon
  - TEM-based, size-specific structure counts
- Include workers with varying ranges of exposure

Epidemiologic studies

A. Cross-sectional study

- Medical exams:
  - Basic physical examination
  - Spirometry and cardiovascular function
- Biological sample collection (blood, sputum)
- Collection of information on other influential factors
- Simultaneous measurement of exposure to CNT and CNF, modified by exposure factors
- Exposure-response analyses

Biomarkers measured in cross-sectional study

Biomarker type	Biomarker*	Rationale
Pulmonary fibrosis	KL-6 glycoprotein MMP1, 7 & 9	KL-6 is early marker of pulmonary fibrosis in workers exposed to some metals; MMPs are involved in degradation of extracellular matrix
Oxidative stress	Myeloperoxidase, SOD, 8-OHdG TNF-α, 8-isoprostane	Mouse analogs elevated with CNT exposure Distinguish local vs. systemic inflammation (with serum markers)
Inflammation	Interleukins (e.g., IL-6 & IL-8), CRP, TNF-α, etc.	Associated with lung cancer, pulmonary fibrosis, and systemic effects in human and animal models.
Coagulant cardiovascular markers	Circulating PAI-1 & fibrinogen, ICAM-1, VCAM-1, PAI-1	Elevated in mice exposed to MWCNT. Also may relate to pulmonary effects.
Neutrophils	Complete blood count with differentials	Increased neutrophils in blood following exposure found in welders

*Markers in sputum, serum, or whole blood

Epidemiologic studies

Cross-sectional Study Status

- Visited 12 primary and secondary CNT and CNF manufacturers and users
- Enrolled 108 participants
- Serum and sputum biomarkers analyzed
- TEM analyses remaining for some sites
- CNT detection in sputum using dark-field microscopy

Planned epidemiologic studies

B: Exposure registry of CNT & CNF workers

- Identify CNT and CNF manufacturers, users and distributors
- Demographic, work history, and exposure information will be requested for employees working with CNT or CNF
- Information will be updated on an ongoing basis

Planned epidemiologic studies

C: Prospective cohort study of CNT & CNF workers

- Use registry to identify cohort members
- Evaluate health outcomes, including pulmonary disease, CVD, cancer
- Methods
  - Periodic questionnaires administered to cohort
  - Linkage of the cohort with disease and mortality registries
  - Development of job-exposure matrix to cover all facilities, workers and time periods

Exposure assessment challenges in epidemiology

- Most-relevant metrics are uncertain
  - Count, mass, surface area, aspect-ratio-specific counts
  - What is implication of inhalable vs. respirable size fraction?
- Signal-to-noise problem: Exposure to ENM is low compared to background ultrafine particulates
  - Non-specific measurement metrics (gravimetric, counts) may give misleading results
  - Emphasis on DRI may detract from adoption of metrics more specific for ENM
  - DRI <u>are</u> useful for indicating exposure to ambient (often, process-derived) ultrafine particulates
- Some filter-based metrics (e.g., electron microscopy) can be expensive
  - Development of representative, task-specific JEMs

Study Collaborators & Support

Field studies

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Measurement methods

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Epidemiology and Exposure Study References

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