**Combined Abstracts from Carbon Nanofiber studies\* and CDC Current Intelligence Bulletin**

**CDC Current Intelligence Bulletin Assessment**

Although data from animal studies with CNF are more limited [Murray et al. 2012; DeLorme et al. 2012], physical-chemical similarities between CNT and CNF and findings of acute pulmonary inflammation and interstitial fibrosis in animals exposed to CNF [Murray et al. 2012] indicate the need to also control occupational exposure to CNF at the REL of 1 µg/m3 EC. Because of uncertainties in the risk estimates some residual risk for adverse lung effects may exist at the REL; therefore, efforts should be made to reduce airborne concentrations to CNT and CNF as low as possible. Until the results from animal research studies can fully explain the mechanisms (e.g., shape, size, chemistry, functionalized) that potentially increase or decrease their toxicity all types of CNT and CNF should be considered a respiratory hazard and occupational exposures controlled at the REL of 1 µg/m3. (<https://www.cdc.gov/niosh/docs/2013-145/pdfs/2013-145.pdf>)

**Abdo 2020 Significant Toxic Effect of Carbon Nanofibers at the Early Stage of Embryogenesis**

Implementation of carbon nanofibers (CNFs) in biomedical applications have successful outcomes, however, they are still considered as a potential hazard. We herein used avian embryos at 3 days and its chorioallantoic membrane (CAM) at 6 days of incubation to evaluate the impact of synthesized CNFs on the early stage of embryogenesis and angiogenesis. **Our data point out that 50 g/embryo concentration of CNFs provoke adverse effects as 75% of CNFs-exposed embryos die within 1–5 days after exposure compared with their matched controls. Furthermore, CNFs significantly inhibit angiogenesis of the CAM after 48-hours post-treatment. Additionally, RT-PCR analysis on seven key controller genes responsible for proliferation, survival, angiogenesis, and apoptosis showed that these genes are deregulated in brain, heart, and liver tissues of CNFs-exposed embryos compared to their matched control.** Our investigation suggests that CNFs could have a toxic effect on the early stages of embryogenesis as well as angiogenesis. Nevertheless, further investigations are required to evaluate the effects of CNFs and elucidate their mechanism on the early stage of the normal development and human health.

**Beard 2018: Carbon nanotube and nanofiber exposure and sputum and blood biomarkers of early effect among U.S. workers**

Background: Carbon nanotubes and nanofibers (CNT/F) are increasingly used for diverse applications. Although animal studies suggest CNT/F exposure may cause deleterious health effects, human epidemiological studies have typically been small, confined to single workplaces, and limited in exposure assessment. Objectives: We conducted an industrywide cross-sectional epidemiological study of 108 workers from 12 U.S. sites to evaluate associations between occupational CNT/F exposure and sputum and blood biomarkers of early effect. Methods: We assessed CNT/F exposure via personal breathing zone, filter-based air sampling to measure background-corrected elemental carbon (EC) (a CNT/F marker) mass and microscopy-based CNT/F structure count concentrations. We measured 36 sputum and 37 blood biomarkers. We used factor analyses with varimax rotation to derive factors among sputum and blood biomarkers separately. We used linear, Tobit, and unconditional logistic regression models to adjust for potential confounders and evaluate associations between CNT/F exposure and individual biomarkers and derived factors. Results: We derived three sputum and nine blood biomarker factors that explained 78% and 67%, respectively, of the variation. **After adjusting for potential confounders, inhalable EC and total inhalable CNT/F structures were associated with the most sputum and blood biomarkers, respectively. Biomarkers associated with at least three CNT/F metrics were 72 kDa type IV collagenase/matrix metalloproteinase-2 (MMP-2), interleukin-18, glutathione peroxidase (GPx), myeloperoxidase, and superoxide dismutase (SOD) in sputum and MMP-2, matrix metalloproteinase-9, metalloproteinase inhibitor 1/tissue inhibitor of metalloproteinases 1, 8-hydroxy-2′-deoxyguanosine, GPx, SOD, endothelin-1, fibrinogen, intercellular adhesion molecule 1, vascular cell adhesion protein 1, and von Willebrand factor in blood, although directions of associations were not always as expected.**

**Dahm 2018 Exposure assessments for a cross-sectional epidemiologic study of US carbon nanotube and nanofiber workers**

Background: Recent animal studies have suggested the potential for wide-ranging health effects resulting from exposure to carbon nanotubes and nanofibers (CNT/F). To date, no studies in the US have directly examined the relationship between occupational exposure and potential human health effects. Objectives: Our goal was to measure CNT/F exposures among US workers with representative job types, from non-exposed to highly exposed, for an epidemiologic study relating exposure to early biologic effects. Methods: 108 participants were enrolled from 12 facilities across the US. Personal, full-shift exposures were assessed based on the mass of elemental carbon (EC) at the respirable and inhalable aerosol particle size fractions, along with quantitatively characterizing CNT/F and estimating particle size via transmission electron microscopy (TEM). Additionally, sputum and dermal samples were collected and analyzed to determine internal exposures and exposures to the hands/wrists. **Results: The mean exposure to EC was 1.00 μg/m3 at the respirable size fraction and 6.22 μg/m3 at the inhalable fraction. Analysis by TEM found a mean exposure of 0.1275 CNT/F structures/cm3, generally to agglomerated materials between 2 and 10 μm. Internal exposures to CNT/F via sputum analysis were confirmed in 18% of participants while ∼70% had positive dermal exposures. Conclusions: We demonstrated the occurrence of a broad range of exposures to CNT/F within 12 facilities across the US. Analysis of collected sputum indicated internal exposures are currently occurring within the workplace. This is an important first step in determining if exposures in the workforce have any acute or lasting health effects.**

**DeLorme 2012: Ninety-Day Inhalation Toxicity Study With A Vapor Grown Carbon Nanofiber in Rats**

A subchronic inhalation toxicity study of inhaled vapor grown carbon nanofibers (CNF) (VGCF-H) was conducted in male and female Sprague Dawley rats. The CNF test sample was composed of > 99.5% carbon with virtually no catalyst metals; Brunauer, Emmett, and Teller (BET) surface area measurements of 13.8 m2 /g; and mean lengths and diameters of 5.8 µm and 158 nm, respectively. Four groups of rats per sex were exposed nose-only, 6 h/day, for 5 days/week to target concentrations of 0, 0.50, 2.5, or 25 mg/m3 VGCF-H over a 90-day period and evaluated 1 day later. Assessments included conventional clinical and histopathological methods, bronchoalveolar lavage fluid (BALF) analysis, and cell proliferation (CP) studies of the terminal bronchiole (TB), alveolar duct (AD), and subpleural regions of the respiratory tract. In addition, groups of 0 and 25 mg/m3 exposed rats were evaluated at 3 months postexposure (PE). Aerosol exposures of rats to 0.54 (4.9 f/cc), 2.5 (56 f/cc), and 25 (252 f/cc) mg/m3 of VGCF-H CNFs produced concentration-related small, detectable accumulation of extrapulmonary fibers with no adverse tissue effects. **At the two highest concentrations, inflammation of the TB and AD regions of the respiratory tract was noted wherein fiber-laden alveolar macrophages had accumulated. This finding was characterized by minimal infiltrates of inflammatory cells in rats exposed to 2.5mg/m3 CNF, inflammation along with some thickening of interstitial walls, and hypertrophy/hyperplasia of type II epithelial cells, graded as slight for the 25mg/m3 concentration. At 3 months PE, the inflammation in the high dose was reduced. No adverse effects were observed at 0.54mg/m3. BALF and CP endpoint increases versus controls were noted at 25mg/m3 VGCF-H but not different from control values at 0.54 or 2.5mg/m3 . After 90 days PE, BALF biomarkers were still increased at 25mg/m3 , indicating that the inflammatory response was not fully resolved. Greater than 90% of CNF-exposed, BALFrecovered alveolar macrophages from the 25 and 2.5mg/m3 exposure groups contained nanofibers (> 60% for 0.5mg/m3 ). A nonspecific inflammatory response was also noted in the nasal passages. The no-observed-adverse-effect level for VGCF-H nanofibers was considered to be 0.54mg/m3 (4.9 fibers/cc) for male and female rats, based on the minimal inflammation in the terminal bronchiole and alveolar duct areas of the lungs at 2.5mg/m3 exposures. It is noteworthy that the histopathology observations at the 2.5mg/m3 exposure level did not correlate with the CP or BALF data at that exposure concentration. In addition, the results with CNF are compared with published findings of 90-day inhalation studies in rats with carbon nanotubes, and hypotheses are presented for potency differences based on CNT physicochemical characteristics. Finally, the (lack of) relevance of CNF for the high aspect ratio nanomaterials/fiber paradigm is discussed.**

**Fraser 2020: Physicochemical characterization and genotoxicity of the broad class of carbon nanotubes and nanofibers used or produced in U.S. facilities**

Abstract Background: Carbon nanotubes and nanofibers (CNT/F) have known toxicity but simultaneous comparative studies of the broad material class, especially those with a larger diameter, with computational analyses linking toxicity to their fundamental material characteristics was lacking. It was unclear if all CNT/F confer similar toxicity, in particular, genotoxicity. Nine CNT/F (MW #1–7 and CNF #1–2), commonly found in exposure assessment studies of U.S. facilities, were evaluated with reported diameters ranging from 6 to 150 nm. All materials were extensively characterized to include distributions of physical dimensions and prevalence of bundled agglomerates. Human bronchial epithelial cells were exposed to the nine CNT/F (0–24 μg/ml) to determine cell viability, inflammation, cellular oxidative stress, micronuclei formation, and DNA double-strand breakage. Computational modeling was used to understand various permutations of physicochemical characteristics and toxicity outcomes **Results: Analyses of the CNT/F physicochemical characteristics illustrate that using detailed distributions of physical dimensions provided a more consistent grouping of CNT/F compared to using particle dimension means alone.** In fact, analysis of binning of nominal tube physical dimensions alone produced a similar grouping as all characterization parameters together. **All materials induced epithelial cell toxicity and micronuclei formation within the dose range tested. Cellular oxidative stress, DNA double strand breaks, and micronuclei formation consistently clustered together and with larger physical CNT/F dimensions and agglomerate characteristics but were distinct from inflammatory protein changes. Larger nominal tube diameters, greater lengths, and bundled agglomerate characteristics were associated with greater severity of effect. The portion of tubes with greater nominal length and larger diameters within a sample was not the majority in number, meaning a smaller percentage of tubes with these characteristics was sufficient to increase toxicity. Many of the traditional physicochemical characteristics including surface area, density, impurities, and dustiness did not cluster with the toxicity outcomes. Conclusion: Distributions of physical dimensions provided more consistent grouping of CNT/F with respect to toxicity outcomes compared to means only. All CNT/F induced some level of genotoxicity in human epithelial cells. The severity of toxicity was dependent on the sample containing a proportion of tubes with greater nominal lengths and diameters.**

**Kisin 2011: Genotoxicity of CNF compared to Asbestos**

The production of carbon nanofibers and nanotubes (CNF/CNT) and their composite products is increasing globally. CNF are generating great interest in industrial sectors such as energy production and electronics, where alternative materials may have limited performance or are produced at a much higher cost. However, despite the increasing industrial use of carbon nanofibers, information on their potential adverse health effects is limited. In the current study, we examine the cytotoxic and genotoxic potential of carbon-based nanofibers (Pyrograf®-III) and compare this material with the effects of asbestos fibers (crocidolite) or single walled carbon nanotubes (SWCNT). The genotoxic effects in the lung fibroblast (V79) cell line were examined using two complementary assays: the comet assay and micronucleus (MN) test. In addition, we utilized fluorescence in situ hybridization to detect the chromatin pan-centromeric signals within the MN indicating their origin by aneugenic (chromosomal malsegregation) or clastogenic (chromosome breakage) mechanisms. **Cytotoxicity tests revealed a concentration- and time-dependent loss of V79 cell viability after exposure to all tested materials in the following sequence: asbestos>CNF>SWCNT. Additionally, cellular uptake and generation of oxygen radicals was seen in the murine RAW264.7 macrophages following exposure to CNF or asbestos but not after administration of SWCNT**. DNA damage and MN induction were found after exposure to all tested materials with the strongest effect seen for CNF. Finally, we demonstrated that CNF induced predominately centromere-positive MN in primary human small airway epithelial cells (SAEC) indicating aneugenic events. Further investigations are warranted to elucidate the possible mechanisms involved in CNF-induced genotoxicity.

**Murray 2012** **Factoring-in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos**

Abstract Background: Carbon nanotubes (CNT) and carbon nanofibers (CNF) are allotropes of carbon featuring fibrous morphology. The dimensions and high aspect ratio of CNT and CNF have prompted the comparison with naturally occurring asbestos fibers which are known to be extremely pathogenic. While the toxicity and hazardous outcomes elicited by airborne exposure to single-walled CNT or asbestos have been widely reported, very limited data are currently available describing adverse effects of respirable CNF. Results: Here, we assessed pulmonary inflammation, fibrosis, oxidative stress markers and systemic immune responses to respirable CNF in comparison to single-walled CNT (SWCNT) and asbestos. **Pulmonary inflammatory and fibrogenic responses to CNF, SWCNT and asbestos varied depending upon the agglomeration state of the particles/fibers. Foci of granulomatous lesions and collagen deposition were associated with dense particle-like SWCNT agglomerates, while no granuloma formation was found following exposure to fiber-like CNF or asbestos.** **The average thickness of the alveolar connective tissue - a marker of interstitial fibrosis - was increased 28 days post SWCNT, CNF or asbestos exposure. Exposure to SWCNT, CNF or asbestos resulted in oxidative stress evidenced by accumulations of 4-HNE and carbonylated proteins in the lung tissues. Additionally, local inflammatory and fibrogenic responses were accompanied by modified systemic immunity, as documented by decreased proliferation of splenic T cells ex vivo on day 28 post exposure. The accuracies of assessments of effective surface area for asbestos, SWCNT and CNF (based on geometrical analysis of their agglomeration) versus estimates of mass dose and number of particles were compared as predictors of toxicological outcomes. Conclusions: We provide evidence that effective surface area along with mass dose rather than specific surface area or particle number are significantly correlated with toxicological responses to carbonaceous fibrous nanoparticles. Therefore, they could be useful dose metrics for risk assessment and management.**

**Shubauer-Berigan 2018 Association of pulmonary, cardiovascular, and hematologic metrics with carbon nanotube and nanofiber exposure among U.S. workers: a cross-sectional study**

Abstract Background: Commercial use of carbon nanotubes and nanofibers (CNT/F) in composites and electronics is increasing; however, little is known about health effects among workers. We conducted a cross-sectional study among 108 workers at 12 U.S. CNT/F facilities. We evaluated chest symptoms or respiratory allergies since starting work with CNT/F, lung function, resting blood pressure (BP), resting heart rate (RHR), and complete blood count (CBC) components. Methods: We conducted multi-day, full-shift sampling to measure background-corrected elemental carbon (EC) and CNT/F structure count concentrations, and collected induced sputum to measure CNT/F in the respiratory tract. We measured (nonspecific) fine and ultrafine particulate matter mass and count concentrations. Concurrently, we conducted physical examinations, BP measurement, and spirometry, and collected whole blood. We evaluated associations between exposures and health measures, adjusting for confounders related to lifestyle and other occupational exposures. **Results: CNT/F air concentrations were generally low, while 18% of participants had evidence of CNT/F in sputum. Respiratory allergy development was positively associated with inhalable EC (p=0.040) and number of years worked with CNT/F (p=0.008). No exposures were associated with spirometry-based metrics or pulmonary symptoms, nor were CNT/F-specific metrics related to BP or most CBC components. Systolic BP was positively associated with fine particulate matter (p-values: 0.015-0.054). RHR was positively associated with EC, at both the respirable (p=0.0074) and inhalable (p=0.0026) size fractions. Hematocrit was positively associated with the log of CNT/F structure counts (p=0.043). Conclusions: Most health measures were not associated with CNT/F. The positive associations between CNT/F exposure and respiratory allergies, RHR, and hematocrit counts may not be causal and require examination in other studies.**

\*bolding added