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

**CDC Current Intelligence Bulletin Assessment [Excerpts; bolding is mine] (**<https://www.cdc.gov/niosh/docs/2013-145/pdfs/2013-145.pdf>)

Carbon nanofibers (CNF), which are structurally similar to MWCNT, have typical diameters approximately 40 to 200 nm [Ku et al. [2006]. CNF have lengths ranging from tens of micrometers to several centimeters, average aspect ratios (length to diameter ratio) of > 100, and they display various morphologies, including cupped or stacked graphene structures. **The primary characteristic that distinguishes CNF from CNT resides in graphene plane alignment. If the graphene plane and fiber axis do not align, the structure is defined as CNF, but when parallel, the structure is considered a CNT [ISO/TS 2008]**.

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.

**The analysis conducted by NIOSH was focused on the types of CNT and CNF included in published research studies. Pulmonary responses were qualitatively similar across the various types of CNT and CNF, purified or unpurified with various metal content, and different dimensions [Lam et al. 2004; Shvedova et al. 2005, 2008; Muller et al. 2005; Ma-Hock et al.** 2009; Pauluhn 2010a; Porter et al. 2010; Mercer et al. 2011; Murray et al. 2012; DeLorme et al. 2012]. The fibrotic lung effects in the animal studies developed early (within a few weeks) after exposure to CNT or CNF, at relatively low-mass lung doses, and persisted or progressed during the post-exposure follow-up (~1–6 months) [Shvedova et al. 2005, 2008; Mercer et al. 2008; Porter et al. 2010; Pauluhn 2010a; Murray et al. 2012]. However, the studied CNT and CNF only represent a fraction of the types of CNT and CNF that are, or will be, in commerce and it is anticipated that materials with different physical and chemical parameters could have different toxicities. At this time, however, given the findings in the published literature, NIOSH recommends that exposures to all CNT and CNF be controlled to less than 1 µg/m3 of respirable elemental carbon as an 8-hr TWA, and that the risk management guidance described in this document be followed. Until results from research can fully explain the physical-chemical properties of CNT and CNF that define their inhalation toxicity, all types of CNT and CNF should be considered a respiratory hazard and exposure should be controlled below the REL.

**Health endpoint studies**

**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.**

**Shvedova 2014Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: one year postexposure comparisons.**

The hallmark geometric feature of single-walled carbon nanotubes (SWCNT) and carbon nanofibers (CNF), high length to width ratio, makes them similar to a hazardous agent, asbestos. Very limited data are available concerning long-term effects of pulmonary exposure to SWCNT or CNF. Here, we compared inflammatory, fibrogenic, and genotoxic effects of CNF, SWCNT, or asbestos in mice 1 yr after pharyngeal aspiration. In addition, we compared pulmonary responses to SWCNT by bolus dosing through pharyngeal aspiration and inhalation 5 h/day for 4 days, to evaluate the effect of dose rate. The aspiration studies showed that these particles can be visualized in the lung at 1 yr postexposure, whereas some translocate to lymphatics. All these particles induced chronic bronchopneumonia and lymphadenitis, accompanied by pulmonary fibrosis. CNF and asbestos were found to promote the greatest degree of inflammation, followed by SWCNT, whereas SWCNT were the most fibrogenic of these three particles. Furthermore, SWCNT induced cytogenetic alterations seen as micronuclei formation and nuclear protrusions in vivo. Importantly, inhalation exposure to SWCNT showed significantly greater inflammatory, fibrotic, and genotoxic effects than bolus pharyngeal aspiration. Finally, SWCNT and CNF, but not asbestos exposures, increased the incidence of K-ras oncogene mutations in the lung. No increased lung tumor incidence occurred after 1 yr postexposure to SWCNT, CNF, and asbestos. Overall, our data suggest that long-term pulmonary toxicity of SWCNT, CNF, and asbestos is defined, not only by their chemical composition, but also by the specific surface area and type of exposure.

**Environmental Studies**

**Chaika 2020 The toxic influence and biodegradation of carbon nanofibers in freshwater invertebrates of the families Gammaridae, Ephemerellidae, and Chironomida**

Carbon nanofibers (CNFs) are widely used in consumer products today. In this study, we assessed the effects of CNFs on the digestive system of three freshwater invertebrate species (Gammaridae, Ephemerellidae, and Chironomidae). The aquatic insects Diamesa sp., Drunella cryptomeria, and Gammarus suifunensis were incubated with the CNFs at the concentration of 100 mg/L during the 7-days period. Histological examination of the whole specimens and the longitudinal sections revealed no toxic effects of CNFs. However, a noticeable change in the structure of the CNFs accumulated in the intestines of the aquatic insects was found by Raman spectroscopy. The registered decrease in the relative proportion of amorphous carbon included in the CNF sample was found in the intestines of Diamesa sp. and D. cryptomeria. The registered effect can indicate a biodegradation of amorphous carbon in the digestive tract of these two insect species. In contrast, the decrease of highly structured carbons and the decrease of G-bonds intensity were registered in the digestive tract of G. suifunensis. **This observation demonstrates the partial biodegradation of CNFs in the digestive tract of G. suifunensis.**

**Guimarães 2021 Multiple toxicity endpoints induced by carbon nanofibers in Amazon turtle juveniles: Outspreading warns about toxicological risks to reptiles**

The toxicity of carbon-based nanomaterials (CNs) has been observed in different organisms; however, little is known about the impact of water polluted with carbon nanofibers (CNFs) on reptiles. Thus, the aim of the current study was to assess the chronic effects (7.5 months) of 1 and 10 mg/L of CNF on Podocnemis expansa (Amazon turtle) juveniles (4 months old) based on different biomarkers. Increased total organic carbon (TOC) concentrations observed in the liver and brain (which suggests CNF uptake) were closely correlated to changes in REDOX systems of turtles exposed to CNFs, mainly to higher nitrite, hydrogen peroxide and lipid peroxidation levels. Increased levels of antioxidants such as total glutathione, catalase and superoxide dismutase in the exposed animals were also observed. The uptake of CNFs and the observed biochemical changes were associated with higher frequency of erythrocyte nuclear abnormalities (assessed through micronucleus assays), as well as with both damage in erythrocyte DNA (assessed through comet assays) and higher apoptosis and necrosis rates in erythrocytes of exposed turtles. Cerebral and hepatic acetylcholinesterase (AChE) increased in turtles exposed to CNFs, and this finding suggested the neurotoxic effect of these nanomaterials. **Data in the current study reinforced the toxic potential of CNFs and evidenced the biochemical, mutagenic, genotoxic, cytotoxic, and neurotoxic effects of CNFs on P. expansa.**

**Gomes 2020 Trophic transfer of carbon nanofibers among eisenia fetida, danio rerio and oreochromis niloticus and their toxicity at upper trophic level**

Although the toxicity of carbon-based nanomaterials has already been demonstrated in several studies, their transfer in the food chain and impact on the upper trophic level remain unexplored. Thus, based on the experimental food chain “Eisenia fetida / Danio rerio / Oreochromis niloticus”, the current study tested the hypothesis that carbon nanofibers (CNFs) accumulated in animals are transferred to the upper trophic level and cause mutagenic and cytotoxic changes. E. fetida individuals were exposed to CNFs and offered to D. rerio, which were later used to feed O. niloticus. The quantification of total organic carbon provided evidence of CNFs accumulation at all evaluated trophic levels. Such accumulation was associated with higher frequency of erythrocyte nuclear abnormalities such as constricted erythrocyte nuclei, vacuole, blebbed, kidney-shaped and micronucleated erythrocytes in Nile tilapia exposed to CNFs via food chain. The cytotoxic effect was inferred based on the smaller size of the erythrocyte nuclei and on the lower “nuclear/cytoplasmic” area ratio in tilapia exposed to CNFs via food chain. **Our study provided pioneering evidence about CNFs accumulation at trophic levels of the experimental chain, as well as about the mutagenic and cytotoxic effect of these materials on O. niloticus.**

**Barrick 2019 Investigating the Impact of Manufacturing Processes on the Ecotoxicity of Carbon Nanofibers: A multi-Aquatic Species Comparison**

Manufactured nanomaterial production is outpacing the ability to investigate environmental hazard using current regulatory paradigms, causing a backlog of materials requiring testing. To ameliorate this issue, regulatory bodies have proposed integrating safety into the production of novel nanomaterials, allowing for hazards to be identified early in development rather than aftermarket release. In addition, there is a growing interest in short‐term ecotoxicity testing to rapidly identify environmental hazards. In this sense, the present study investigated 3 carbon nanofibers (CNFs), created with different production methods, using short‐term in vitro and in vivo exposures on fish cell lines, mussel hemocytes, crustacea, and algae. The present study investigated if differences in ecotoxicity hazard between the CNFs could be identified and, if so, which product could be considered less hazardous. A major challenge in assessing the potential hazards posed by manufactured nanomaterials is standardizing the preparation for testing. Standardized operating protocols have been proposed using protein to facilitate the preparation of stable stock suspension, which is not environmentally representative. As such, the study also assessed the potential impacts these standardized protocols (with or without the use of protein) could have on the interpretation of environmental hazard. **The results demonstrated that there were clear differences between the 3 CNFs and that the dispersion protocol influenced the interpretation of hazard, demonstrating a need for caution when interpreting ecotoxicity in a regulatory context.**

**Montalvao 2019 Carbon nanofibers are bioaccumulated in Aphylla williamsoni (Odonata) larvae and cause REDOX imbalance and changes of acetylcholinesterase activity**

Carbon-based materials have been considered very promising for the technological industry due to their unique physical and chemical properties, namely: ability to reduce production costs and to improve the efficiency of several products. However, there is little information on what is the level of exposure that leads to adverse effects and what kind of effects is expected in aquatic biota. Thus, the aim of the present study was to evaluate the toxicity of carbon nanofibers (CNFs) in dragonfly larvae (Aphylla williamsoni) based on predictive oxidative-stress biomarkers, antioxidant activity reduction and neurotoxicity. After ephemeral models' exposure to CNFs (48 h; at 500 μg/L), data have shown that these pollutants did not change larvae's nutritional status given the concentration of total soluble carbohydrates, total proteins and triglycerides in them. However, the levels of both nitric oxide and substances reactive to thiobarbituric acid (lipid peroxidation indicators) have increased and the antioxidant activity based on total thiol levels and on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (%) has reduced, and **it suggests REDOX imbalance induction by CNFs**. In addition, **larvae exposed to these pollutants showed significant acetylcholinesterase activity reduction in comparison to the control group.** Thus, the present study has brought further knowledge about how carbon-based materials can affect benthic macroinvertebrates and emphasized their ecotoxicological potential in freshwater environments.

**\*bolding added**