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

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Abstract

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.

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Conflict of interest

The authors declare they have no competing financial interests.

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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.

Conclusions—Inhalable rather than respirable CNT/F was more consistently associated with fibrosis, inflammation, oxidative stress, and cardiovascular biomarkers.

Keywords

biomarker; blood; carbon nanotube; carbon nanofiber; occupational; sputum

1. Introduction

Carbon nanotubes (CNT) and carbon nanofibers (CNF; collectively CNT/F) are increasingly used in many novel applications spanning several products and industries such as manufacturing, electronics, information technology, healthcare, and life sciences (National Institute for Occupational Safety and Health, 2013; Schubauer-Berigan et al., 2011). Although the number of workers involved in the manufacture and use of CNT/F is relatively small, it is growing steadily (Schubauer-Berigan et al., 2011). In addition, recent exposure assessment studies have documented exposure to CNT/F among workers who manufacture these materials or use them in downstream applications including incorporation in semiconductors, polymers, composites, or other products (Debia et al., 2016; Guseva Canu et al., 2016).

There is concern regarding possible health effects of exposure to CNT/F because of their physical similarities to workplace or environmental particulate matter and asbestos (National Institute for Occupational Safety and Health, 2013; Oberdörster et al., 2015). Workplace and environmental exposure to airborne particulate matter such as diesel emissions and ultrafine air pollution has been associated with cancer, cardiovascular and respiratory diseases, and eventual mortality (Brook et al., 2010; International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012, 2016; Kuempel et al., 2017; U.S. Environmental Protection Agency, 2009). CNF and a few types of multi-walled CNT (MWCNT; Mitsui-7 and similar materials) are physically similar to asbestos. Kasai et al. (2016) found that one type of MWCNT (MWCNT-7, Mitsui Ltd., Japan) is carcinogenic to the lungs of rats. In addition, the International Agency for Research on Cancer (IARC) classified MWCNT-7 as possibly carcinogenic (IARC Group 2B) to humans (Grosse et al., 2014; Kuempel et al., 2017). Evidence for the carcinogenicity of single-walled CNT and other types of MWCNT was insufficient (Grosse et al., 2014; Kuempel et al., 2017), but other potential adverse effects are a concern (Aragon et al., 2017; Erdely et

al., 2011; Kuempel et al., 2017; National Institute for Occupational Safety and Health, 2013).

The health effects of CNT/F are difficult to assess as a general class because of the diversity of these materials. Toxicity may be affected by their different physical and chemical properties such as their shape, aspect ratio, size, structure, surface area and chemistry, number of particles for a given mass, purity, functionalization, and tendency to agglomerate (National Institute for Occupational Safety and Health, 2013; Oberdörster et al., 2015). Animal studies suggest that exposure to CNT/F may cause oxidative stress and fibrotic, inflammatory, and cardiovascular health effects locally and systemically (Erdely et al., 2009; Oberdörster et al., 2015). The few human epidemiological studies that have been conducted, however, have typically been small, confined to single workplaces, and limited in exposure assessment (Fatkhutdinova et al., 2016; Kuijpers et al., 2018; Lee et al., 2015; Liou et al., 2015; Shvedova et al., 2016; Vlaanderen et al., 2017). Therefore, we conducted an industrywide cross-sectional epidemiological study of 108 workers from 12 sites across the U.S. to evaluate associations between occupational exposure to CNT/F and human health effects (Schubauer-Berigan et al., unpublished results¹).

The latencies for overt health effects such as pulmonary fibrosis, other respiratory diseases, cardiovascular diseases, cancer, or other chronic diseases are likely several years or decades. The majority of workers included in our study likely have not been exposed to CNT/F long enough or at high enough levels to cause overt health effects at this time. For these reasons, we evaluated relationships between occupational CNT/F exposure and early sputum and blood biomarker responses and patterns. We included biomarkers that have been positively associated with pulmonary fibrosis or that were increased in animal models of CNT/F exposure, pulmonary toxicant exposure studies, and lung cancer (Brook et al., 2010; Erdely et al., 2009, 2011; Ichiyasu et al., 2012; Liou et al., 2012; Pine et al., 2011). Virtually all of the biomarkers we evaluated, however, have no known clinical significance. Therefore, associations we observed between CNT/F exposure and biomarkers may reflect short-term reactions and may not indicate future chronic diseases. Nevertheless, evaluating biomarkers may be useful for elucidating possible mechanisms for CNT/F exposure or disease pathways, especially when compared to animal studies. For the current analysis, we focused on biomarkers of fibrosis, inflammation, oxidative stress, and cardiovascular effects.

2. Materials and methods

2.1. Study population

We have previously described how we identified companies manufacturing, using, or distributing CNT/F (Dahm et al., 2011; Schubauer-Berigan et al., 2011). Briefly, we initially identified companies via industry profiles (Dun & Bradstreet, Inc., 2016; Lux Research, Inc., 2006, 2007), internet searches, and personal contacts or colleagues (Dahm et al., 2011; Schubauer-Berigan et al., 2011). Of 139 companies we identified in 2008 that worked with

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¹Citations to “Schubauer-Berigan et al., unpublished results” refer to a journal article manuscript we wrote that is currently under review for publication. The current citation is Schubauer-Berigan, M.K., Dahm M.M., Erdely, A., Beard, J.D., Birch, M.E., Evans, D.E., et al. Association of pulmonary, cardiovascular, and hematologic metrics with carbon nanotube and nanofiber exposure among U.S. workers: a cross-sectional study.

various types of engineered carbonaceous nanomaterials, 59 met the inclusion criteria of manufacturing, using, or distributing CNT/F in the U.S. at full-, pilot-, or research-scale (Dahm et al., 2011; Schubauer-Berigan et al., 2011) (Figure 1). We included companies at research-scale only if they had plans to scale up within 5 years (Dahm et al., 2011; Schubauer-Berigan et al., 2011). We identified an additional seven eligible companies in 2012 by updating the original search (Figure 1). The eligible companies employed over 500 workers who work with CNT/F (Figure 1). For the current cross-sectional epidemiological study, we visited the facilities of 12 companies between December 2012 and September 2014, and 108 workers participated (75% of the 144 invited workers) (Figure 1).

The Human Subjects Review Board of the U.S. Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health (NIOSH), approved this study. All workers provided written informed consent after the study was explained to them.

2.2. Sputum and blood collection and biomarker assessment

Workers underwent a limited in-person clinical exam conducted by a physician at the middle or end of the midweek shift for which we collected their personal breathing zone exposure measurements (see Section 2.3) (Schubauer-Berigan et al., unpublished results). As part of this exam, we collected induced sputum from each worker. We followed the procedure outlined in an induced sputum evaluation among popcorn manufacturing workers (Akpınar-Elci et al., 2005), but modified to use isotonic saline at lower nebulizer output rate. This modification reduces the occurrence of side effects while producing sputum of acceptable quality (Loh et al., 2004). Five workers had contraindications for sputum induction and were ineligible (Figure 1). In addition, five workers declined to provide sputum samples and we did not try sputum induction for six workers due to complications with spirometry (two workers), phlebotomy (one worker), or an electrical power loss (three workers) (Figure 1). One worker participated in sputum induction but did not produce any sputum and one worker started sputum induction, but decided to stop before a sufficient amount could be collected (Figure 1).

The remaining 90 eligible workers (Figure 1) inhaled a sterile isotonic saline solution produced by a compressed-air generated nebulizer for 12 minutes total. Workers breathed through the mouthpiece and then, every two minutes, removed the mouthpiece, spit saliva into a cup, took a deep breath through the mouthpiece, and then coughed the full breath along with sputum into a sputum cup.

After collecting sputum, we treated it with a mucolytic agent (Sputolysin® or dithiothreitol, Sigma-Aldrich Co. LLC., St. Louis, MO) and incubated it for 15 minutes in a shaking water bath set at 37°C. We then centrifuged the sputum at approximately 500 × g and separated it into acellular and cellular fractions within one hour of collection. We re-suspended the cellular fraction in an alcohol-based fixative/cryoprotectant (Sacomanno fluid or methanol, Sigma-Aldrich Co. LLC., St. Louis, MO) using at least a 50% preservative concentration. We split the acellular fraction into aliquots and stored it at −20°C in the field. We stored the cellular fraction at 4°C in the field. Samples were shipped overnight and stored at −80°C according to National Cancer Institute's (NCI) best practices guidelines (National Cancer Institute, 2016). In total, we collected and stored sputum samples for 90 workers (Figure 1).

We used the cellular fraction of each sputum sample to assess its quality and deemed samples to be of acceptable quality if squamous epithelial cells comprised <20% of the total cells in the sample (Fireman et al., 2010; Spanevello et al., 2000). Of the 90 sputum samples collected, 1 (1%) had <20% squamous epithelial cells, indicating sputum quality was poor overall, and that much of the specimen collected may have originated from the upper respiratory tract and oral cavity rather than the lower airways. Reasons for the poor sputum quality may include that we used isotonic rather than hypertonic saline to reduce the occurrence of side effects for workers during sputum production. We also used a compressed-air nebulizer (the only model we could find available) and a lower nebulizer output rate. These modifications, perhaps along with processing specimens in the field, probably reduced the quality of sputum we obtained (Loh et al., 2004). A recent study, however, found sputum differential cell count reproducibility is not affected by the presence of high percentage of squamous epithelial cells (Sohani et al., 2011). Whether sputum biomarker level reproducibility is affected by the presence of high percentage of squamous epithelial cells remains unknown.

We also collected 44 mL of whole blood from workers' arms of which 27 mL were used to measure biomarkers for the current analysis. Five workers declined to provide blood samples and we stopped phlebotomy for one worker before a sufficient amount could be collected (Figure 1). For the remaining 102 workers (Figure 1), we first collected two 5 mL serum separator tubes for serum. We also collected two 8.5 mL EDTA tubes for plasma. After collection, we inverted the serum tubes five times and gave the blood 30 minutes to clot. We inverted plasma tubes 10 times and immediately spun them down. The immediate spin was recommended by the manufacturer of the biomarker kits for oxidative stress biomarkers. We spun all serum and plasma samples to be fractionated at 1000 g for 10 minutes at 4°C. We split the samples into aliquots and stored them in the field at -20°C. Samples were shipped overnight and stored at -80°C according to NCI's best practices guidelines (National Cancer Institute, 2016). In total, we collected and stored blood samples for 102 workers (Figure 1).

We shipped aliquots of sputum (acellular fraction) and plasma overnight to a contractor (Myriad RBM, Inc., Austin, TX) for measurement of biomarkers using a custom designed panel. The contractor measured 30 and 31 biomarkers of fibrosis, inflammation, oxidative stress, and cardiovascular effects in sputum and plasma, respectively, via multiplexed immunoassays (Tables S1 and S2) (Welsh and Mapes, 2016). We also measured an additional six biomarkers in sputum, five in plasma, and one in serum (mucin-1/Krebs von den Lungen-6; MUC-1/KL-6) in-house at NIOSH, Morgantown, WV, via ELISA for a total of 36 and 37 biomarkers measured in sputum and blood (serum and plasma), respectively (Tables S1 and S2). All samples were run with blank replicates and the coefficient of variation of replicate samples was 30% (Welsh and Mapes, 2016). The frequencies and percentages of sputum and blood biomarkers' values measured above the least detectable dose (LDD) are shown in Tables S1 and S2, respectively. We excluded four sputum and eight blood biomarkers that had less than or equal to 10% of values measured above the LDD from further analyses.

2.3. Exposure assessment

We have described the methods we used to assess exposure to CNT/F elsewhere (Dahm et al., 2018), but followed previous exposure assessment studies (Birch et al., 2011; Dahm et al., 2012, 2013, 2015; Evans et al., 2010). Briefly, we collected three types of full-shift, filter-based, personal breathing zone (PBZ) air samples for each worker over two midweek days (we collected workers' blood and sputum on one of these days) to measure (1) elemental carbon (EC), a marker of CNT/F, at the inhalable fraction, (2) EC at the respirable fraction, and (3) CNT/F exposure, determined via transmission electron microscopy (TEM), at the inhalable fraction. We also concurrently collected two types of full-shift, filter-based, background air samples over two days, so that EC measurements in the workplace could be background-corrected, to measure (1) inhalable EC and (2) respirable EC. Background correction was necessary because the EC exposure measurements we collected in our study could also reflect other anthropogenic sources of fine and ultrafine EC present at the facilities and, therefore, may not be specific to CNT/F exposure (Birch et al., 2011).

After we collected PBZ and background air samples, we analyzed the EC samples using a modified NIOSH Manual of Analytical Methods (NMAM) 5040 (Birch et al., 2011, 2016; Dahm et al., 2012, 2015, National Institute for Occupational Safety and Health, 2003). We calculated inhalable and respirable EC exposure in units of $\mu\text{g}/\text{m}^3$ of air for each worker per day sampled.

We analyzed air samples by microscopy using a modified NMAM 7402 Asbestos by TEM (National Institute for Occupational Safety and Health, 1994) as discussed elsewhere (Dahm et al., 2012, 2015). A detailed description of this procedure can be found in a new NMAM Chapter (Birch et al., 2017). Briefly, we counted the number of CNT/F structures on three small filter sections transferred to TEM grids. Specifically, the number of CNT/F structures in 40 randomly selected grid openings (total) were counted. We defined a CNT/F structure as either a single CNT/F or, more commonly, as an agglomerate of multiple CNT/F. We also determined whether the structure was a single CNT/F or, if the structure was an agglomerate, whether the length of its longest dimension was less than 1, 2, 5, and 10 μm . We calculated air concentrations of CNT/F structures in size classes consisting of single CNT/F, <1 μm , <2 μm , <5 μm , <10 μm , and total inhalable structures per cm^3 . We also created a dichotomous variable for any inhalable CNT/F exposure based on whether we detected via TEM any CNT/F in workers' microscopy air samples.

We utilized three direct-reading instruments consisting of a condensation particle counter (CPC 3007; TSI, Inc., Shoreview, MN), an electrical low-pressure impactor (ELPI; Dekati, Ltd, Tampere, Finland), and a combined photometer/optical particle counter (DustTrak DRX Model 8533; TSI, Inc., Shoreview, MN), operating simultaneously and in parallel, on an aerosol sampling cart (Dahm et al., 2013, 2018; Evans et al., 2010). We used the direct-reading instruments as general area samplers within each workplace to measure full-shift exposure over two days to ultrafine and fine particulates (i.e., not confined to CNT/F) from occupational or environmental sources. We measured particle number concentrations in the size range of 10 to 1,000 nm with the CPC, particle number concentrations in the size range of 22.9 to 95.8 nm with the ELPI, and particulate matter mass with an aerodynamic diameter 2.5 μm ($\text{PM}_{2.5}$) with the DustTrak. Although the direct-reading instrument data were

collected from various locations as area samples around the facilities visited for the current study, we used information from direct observations and worker interviews regarding work shifts and tasks to assign representative time series of direct-reading instrument data for each worker per day sampled (Dahm et al., 2018). We considered measurements from the direct-reading instruments as exposure variables and as potential confounders of associations between the CNT/F exposure variables and the biomarkers (see Section 2.5).

We also evaluated the presence of CNT/Fin workers' sputum samples using dark -field microscopy (Dahm et al., 2018; Mercer et al., 2011; Schubauer-Berigan et al., unpublished results). To identify CNT/F in the cellular matrix of the sputum, we prepared a cytospin of the cellular fraction of each sputum sample on ultrasonically cleaned, laser cut slides (Schott North America, Inc, Elmsford, NY).

We used information on occupational history ascertained via standardized in-person interviews (see Section 2.4) to create a variable for duration (years) of exposure to CNT/F in current and past jobs (Dahm et al., 2018; Schubauer-Berigan et al., unpublished results). We also used information from direct observations of workers and worker interviews regarding work shifts and tasks to develop a variable for job title (Dahm et al., 2018). For this variable, we classified workers into one of the following job titles: administrator, chemist, engineer, maintenance, research and development, technician.

2.4. Covariate assessment

We collected information on demographics, lifestyle, medical history, medications, and occupational history via standardized, computer-assisted, in-person interviews (Schubauer-Berigan et al., unpublished results). We also obtained clinical measures via standardized in-person exams (Schubauer-Berigan et al., unpublished results).

For medications, we collected information on current prescription and non-prescription medication use in an open-ended manner (Schubauer-Berigan et al., unpublished results). For the current analysis, we considered only use of non-steroidal anti-inflammatory drugs (NSAID) and medications used to treat cholesterol and hypertension.

As described by Schubauer-Berigan et al. (unpublished results), we used information from standardized in-person interviews and exams to create a modified cardiovascular health metric (CHM) score (based on the American Heart Association's "Life's Simple Seven" index [Lloyd-Jones et al., 2010]). For each worker, we assigned one point each for body mass index $<25 \text{ kg/m}^2$, waist circumference $<102 \text{ cm}$ (men) or 88 cm (women), not being a current cigarette smoker (at least 100 lifetime and smoked within the past six months), not ever being told by a doctor that he/she had high blood pressure, not currently taking any prescription or non-prescription cholesterol medication, and not ever had diabetes. We summed scores to obtain total CHM scores between zero and six.

Information we collected as part of the occupational history included regular work shift, regular shift hours (start, stop), and hours per week usually worked for the current job (Schubauer-Berigan et al., unpublished results). For current and past jobs in which workers were exposed to CNT/F, chemicals, dusts, or fumes, we also collected information on

company name and location, department, job title, start and stop dates of work, activities and duties, and any exposure to CNT/F, chemicals, dusts, or fumes. For the current analysis, we had sufficient exposed workers to consider current exposure to dusts (i.e., carbon powder, diesel exhaust, heavy smoke, silica, and other dusts), other nanomaterials (i.e., not CNT/F), polymers, solvents, strong acids, and strong bases. We also considered past exposure to dusts, fumes, lead, metalworking, other nanomaterials, paint, polymers, solvents, strong acids, and strong bases.

2.5. Statistical analyses

For the four sputum and five blood biomarkers that had more than 90%, but less than 100%, of values measured above the LDD, we imputed values below the LDD as one-half the LDD (Table S4). We did not impute values measured below the LDD for the remaining 16 sputum and two blood biomarkers that had values measured below the LDD (Table S4).

We calculated the arithmetic mean of the two days of available air sample results for inhalable EC, respirable EC, inhalable CNT/F structures, particle (10 to 1,000 nm) number concentration, particle (22.9 to 95.8 nm) number concentration, and PM_{2.5}. We also calculated the arithmetic mean of the two days of available air sample results for inhalable single CNT/F structures and inhalable CNT/F structures less than 1, 2, 5, and 10 µm. We combined the engineer and maintenance job titles because there were fewer than five maintenance workers.

We conducted analyses for sputum and blood biomarkers separately. We conducted two primary analyses to:

1. identify patterns among biomarkers via exploratory factor analyses with varimax rotation and then evaluate, individually, associations between derived factor scores and 12 CNT/F and three direct-reading instrument exposure metrics (see Table S3 for a list of these metrics) via regression models and
2. evaluate associations between individual biomarkers and 12 CNT/F and three direct-reading instrument exposure metrics via regression models.

We used factor analysis because biomarker responses to exposures are not likely to occur in isolation (i.e., if exposure has any effect on biomarkers, then the levels of more than one biomarker are likely to change after exposure). Factor analysis harnesses the correlations among biomarkers and derives new variables, or factors, that are linear combinations (i.e., weighted sums) of individual biomarkers (Gorsuch, 1983). Therefore, evaluating associations between CNT/F exposure and the derived biomarker factors may be a more accurate reflection of reality than evaluating associations between CNT/F exposure and individual biomarkers. Despite this, our second primary analysis evaluated associations between CNT/F exposure and individual biomarkers to aid in our interpretation of associations between CNT/F exposure and biomarker factors.

For both primary analyses, we explored transforming biomarker values via Box-Cox transformations to improve the normality of the biomarker data and to meet assumptions for factor analysis and linear regression models (Box and Cox, 1964). A natural logarithm

transformation was the best choice for the majority of biomarkers. We performed exploratory factor analyses using the natural logarithm transformed values of 16 sputum biomarkers (four of which included imputed values) and 27 blood biomarkers (five of which included imputed values) (Table S4). We retained a factor if it had an eigenvalue greater than one. We evaluated associations between derived factor scores and 12 CNT/F and three direct-reading instrument exposure metrics using linear regression models adjusted for potential confounders.

For the second primary analysis, we evaluated associations between 12 CNT/F and three direct-reading instrument exposure metrics and appropriately transformed values of 32 individual sputum and 29 blood biomarkers using regression models adjusted for potential confounders. For sputum biomarkers, we used linear regression models for 12 biomarkers, Tobit regression models for 16 biomarkers, and unconditional logistic regression models for four biomarkers (Table S4). For blood biomarkers, we used linear regression models for 22 biomarkers, Tobit regression models for six biomarkers, and unconditional logistic regression models for one biomarker (Table S4). The outcome for the unconditional logistic regression model analyses was one if the biomarker value was greater than or equal to the LDD and zero otherwise (Table S4).

For both primary analyses, we considered linear, natural logarithm, and categorical (e.g., five categories) terms to model nine continuous CNT/F and three direct-reading instrument exposure variables. Using the Akaike Information Criterion (AIC), we determined that linear or natural logarithm terms were almost always the best ways to model these exposures because they almost always had the lowest AICs (Akaike, 1974; Howe et al., 2011).

To determine the potential confounders to include in our regression models, we considered potential confounders' likely associations with CNT/F exposure and the biomarkers. We also considered potential confounders' correlations with each other, which we determined via Spearman correlation coefficients, and variance inflation factors. We used the mean squared error to compare models that included a certain potential confounder with models that excluded it (Greenland, 2008; Winer, 1978). After considering several adjustment sets that included a wide variety of demographic, lifestyle, medical history, medication, occupational toxicant, and direct-reading instrument covariates, we settled on adjusting for age at interview/exam (continuous linear term in unrounded years), sex, race/ethnicity (non-Hispanic White, other), level of schooling (high school graduate, vocational school, or some college; college graduate or postgraduate), and CHM score (0–4, 5–6).

We considered linear, natural logarithm, and categorical (e.g., five categories) terms (Howe et al., 2011), as appropriate, to model continuous potential confounders (e.g., age at interview/exam, race/ethnicity, level of schooling, CHM score, etc.). We used the AIC to determine how to model continuous potential confounders (Akaike, 1974; Howe et al., 2011).

For sensitivity analyses, we repeated analyses using the maximum of the two days of available air sample results for inhalable EC, respirable EC, and inhalable CNT/F structures.

We also conducted analyses not adjusted for any potential confounders and adjusted for different or additional potential confounders.

We performed all analyses with SAS version 9.4 (SAS Institute Inc., Cary, NC, 2013).

3. Results

The median age of the 103 workers who provided blood or sputum was 45 years and there were two modes, among workers in their 30s and 50s, in the age distribution (Table 1). Workers were predominantly male, non-Hispanic White, and college graduates (Table 1). The majority of workers had unhealthy body mass indexes, although fewer had unhealthy waist circumferences (Table 1). Fifteen percent of workers were current smokers and the majority currently drank alcohol (Table 1). Relatively few workers had a current cold, asthma, heart trouble, or diabetes, but 28% had ever been told they had high blood pressure (Table 1). Percentages of workers currently taking NSAID or medications to treat cholesterol or hypertension were 26, 14, and 16, respectively (Table 1). Just over half of workers had CHM scores (i.e., 5 or 6) that reflected low cardiovascular disease risk and almost all workers' regular work shift was days (Table 1). Summary statistics for sputum and blood biomarkers and for CNT/F and direct-reading instrument exposure variables are provided in the Supplementary data (Tables S1, S2, and S3).

About half of the biomarkers had a lower AIC for inhalable EC than for respirable EC, whereas this pattern was reversed for the other half of the biomarkers (not shown). Among the inhalable CNT/F structure metrics, total CNT/F structures had the lowest AIC for about a fourth of the biomarkers followed by single CNT/F structures and CNT/F structures $<1\text{ }\mu\text{m}$, which each had the lowest AIC for about a fifth of the biomarkers (not shown). For the direct-reading instrument metrics, ELPI had the lowest AIC for about half of the biomarkers (not shown).

3.1. Sputum biomarkers

Spearman correlation coefficients showed associations among sputum biomarkers were almost all positive with magnitudes (in absolute value) ranging from <0.01 (apolipoprotein A-II [Apo-AII] and interleukin-18 [IL-18]) to 0.98 (apolipoprotein A-I [Apo A-I] and Apo A-II) (Excel File Table S6). The factor analysis derived three factors that explained 78% of the variance (Table 2). Ten biomarkers from all four *a priori* groups (i.e., fibrosis, inflammation, oxidative stress, cardiovascular) loaded onto factor 1, whereas only four biomarkers from two *a priori* groups loaded onto factor 2 and two biomarkers from two *a priori* groups loaded onto factor 3 (Table 2).

After adjusting for potential confounders, inhalable EC had a significant positive association with sputum factor 1 (Table 3). Total inhalable CNT/F structures and inhalable CNT/F structures $<5\text{ }\mu\text{m}$ and $<10\text{ }\mu\text{m}$ had significant inverse associations with factor 3 (Table 3).

For individual sputum biomarkers, inhalable EC had significant positive associations with 72 kDa type IV collagenase/matrix metalloproteinase-2 (MMP-2), osteopontin/secreted phosphoprotein 1 (SPP-1), metalloproteinase inhibitor 1/tissue inhibitor of

metalloproteinases 1 (TIMP-1), interleukin-6 receptor subunit beta (IL-6R- β), 8-hydroxy-2'-deoxyguanosine (8-OHdG), superoxide dismutase (SOD), fibrinogen, and vascular cell adhesion protein 1 (VCAM-1) (Excel File Table S7). Respirable EC had a significant positive association with SOD (Excel File Table S7). At least one of the six inhalable CNT/F structures metrics was significantly associated with interstitial collagenase/matrix metalloproteinase-1 (MMP-1; positive), interleukin-8 (IL-8; inverse), IL-18 (inverse), glutathione peroxidase (GPx; inverse), myeloperoxidase (MPO; inverse), and SOD (positive) with total CNT/F structures and CNT/F structures <2 μ m, <5 μ m, and <10 μ m each having the most (four) significant associations (Excel File Table S7). At least one of the three direct-reading instrument metrics had significant positive associations with MMP-2, matrilysin/matrix metalloproteinase-7 (MMP-7), matrix metalloproteinase-9 (MMP-9), SPP-1, TIMP-1, IL-6R- β , and GPx with ELPI having the most (five) significant associations (Excel File Table S7). Job title was significantly associated with 8-OHdG (administrator vs. technician: positive) and SOD (administrator, chemist or research and development, engineer or maintenance vs. technician: inverse) (Excel File Table S7 and Excel File Table S8). Any CNT/F had significant inverse associations with alpha-2-macroglobulin (α -2-M), tumor necrosis factor alpha (TNF- α), and GPx, whereas CNT duration had significant positive associations with TNF- α and SOD (Excel File Table S7).

3.2. Blood biomarkers

There was a mixture of positive and inverse correlations among blood biomarkers with magnitudes (in absolute value) ranging from <0.01 (C-reactive protein [CRP] and C-C motif chemokine 22/macrophage derived chemokine [MDC]) to 0.56 (TIMP-1 and plasminogen activator inhibitor 1 [PAI-1]) (Excel File Table S9). The factor analysis for blood biomarkers derived nine factors that explained 67% of the variance (Table 4). Eight of the nine factors contained two to five biomarkers from at least two *a priori* groups, whereas factor 6 contained two biomarkers from only one *a priori* group (Table 4).

After adjusting for potential confounders, inhalable EC had significant inverse associations with factors 6 and 9 (Table 5). Respirable EC had significant inverse associations with factors 3, 6, and 9 (Table 5). At least two of the six inhalable CNT/F structures metrics were significantly associated with factors 1 (positive), 3 (positive), 5 (positive), 6 (inverse), and 9 (inverse) with total CNT/F structures and CNT/F structures <10 μ m each having the most (five) significant associations (Table 5). At least one of the three direct-reading instrument metrics was significantly associated with factors 1 (positive), 2 (positive), 3 (inverse), 6 (inverse), 7 (positive), and 8 (positive) with ELPI having the most (four) significant associations (Table 5). Job title had a significant inverse association with factor 5 (research and development vs. technician) (Table 5). Any CNT/F was significantly associated with factors 1 (positive), 3 (positive), 4 (positive), and 7 (inverse), whereas CNT duration had a significant inverse association with factor 9 (Table 5).

When we considered blood biomarkers individually, inhalable EC was significantly associated with α -2-M (positive), Apo-AI (positive), 8-OHdG (positive), GPx (positive), and von Willebrand factor (vWF; inverse) (Excel File Table S10). Respirable EC was significantly associated with Apo-AI (positive), Apo-AII (inverse), GPx (positive), and vWF

(inverse) (Excel File Table S10). At least one of the six inhalable CNT/F structures metrics was significantly associated with MMP-9 (positive), TIMP-1 (positive), α -2-M (positive), complement C3 (C3; inverse), GPx (positive), SOD (inverse), endothelin-1 (EDN1; positive), fibrinogen (total: inverse; single, $<1\ \mu\text{m}$, and $<2\ \mu\text{m}$: positive), intercellular adhesion molecule 1 (ICAM-1; positive), tissue-type plasminogen activator (t-PA; inverse), VCAM-1 (positive), and vWF (inverse) with total CNT/F structures having the most (10) significant associations (Excel File Table S10). At least one of the three direct-reading instrument metrics was significantly associated with MUC-1/KL-6 (positive), MMP-2 (inverse), MMP-7 (positive), TIMP-1 (positive), CRP (positive), IL-6R- β (positive), IL-8 (inverse), 8-OHdG (inverse), GPx (positive), MPO (positive), EDN1 (inverse), and PAI-1 (positive) with ELPI having the most (nine) significant associations (Excel File Table S10). Job title was significantly associated with MMP-2 (chemist, research and development vs. technician: inverse), MMP-9 (administrator vs. technician: inverse), IL-18 (chemist vs. technician: positive), GPx (administrator vs. technician: inverse), and SOD (research and development vs. technician: positive) (Excel File Table S10 and Supplementary data, Table S5). Any CNT/F was significantly associated with TIMP-1 (positive), Apo-AII (positive), C3 (inverse), IL-8 (inverse), 8-OHdG (positive), GPx (inverse), and VCAM-1 (positive), whereas CNT duration was significantly associated with MMP-2 (positive), eotaxin-1 (inverse), and GPx (positive) (Excel File Table S10).

Associations between CNT/F exposure and sputum or blood factors or biomarkers were generally similar when we repeated analyses using the maximum rather than the mean of the two days of available air sample results for inhalable EC, respirable EC, and inhalable CNT/F structures (not shown). Results were also generally similar when we did not adjust for any potential confounders or when we adjusted for different or additional potential confounders (not shown).

4. Discussion

In this industrywide cross-sectional epidemiological study of U.S. workers exposed to CNT/F, sputum biomarkers were more strongly correlated with each other than were blood biomarkers. Consequently, fewer factors were derived that explained more variation among sputum biomarkers than among blood biomarkers. Although we observed some associations between CNT/F metrics and sputum inflammation or cardiovascular biomarkers, CNT/F exposure was more consistently associated with sputum fibrosis and oxidative stress biomarkers than inflammation or cardiovascular biomarkers. In blood, we observed associations between CNT/F exposure and biomarkers from all four *a priori* groups. For both sputum and blood biomarkers, associations varied by CNT/F and direct-reading instrument metric. In addition, although there was not a clear best fitting EC or CNT/F structure metric, ELPI was typically the best fitting direct-reading instrument metric.

Our study is the first to use factor analysis to derive patterns of biomarker responses and to evaluate relationships between CNT/F exposure and biomarker factors. Interestingly, biomarkers did not load onto factors that corresponded to *a priori* groups. Sputum biomarkers were also more highly correlated with each other than blood biomarkers, which resulted in fewer sputum factors that explained more of the variance relative to blood factors.

This may have been related to the “dilution” of sputum by saliva, which could have differed among study participants, because biomarker concentrations may have been higher or lower together in samples that were more or less diluted, which would have led to high correlation among individual biomarker measurements and fewer factors. However, it is unlikely that this would cause a spurious association with CNT/F exposure. Associations we observed between CNT/F exposure and sputum factors generally agreed with those we observed between CNT/F exposure and individual sputum biomarkers, but this was less true for blood factors and biomarkers. While this may suggest factor analysis may not have been as useful for blood biomarkers as for sputum biomarkers, the nine factors needed to explain the variance of circulating biomarkers may suggest a lack of a consistent measurable effect of exposure as changes were not always directionally adverse.

Previous studies may give some insights into the formation of some of the factors derived in our study. For example, in a study of humans, Guiot et al. (2017) found MUC-1/KL-6, MMP-7, and IL-8, which all loaded onto sputum factor 2 in our study, were all elevated in the sputum of 15 idiopathic pulmonary fibrosis patients compared to 30 health controls. In another study of humans, Przepiera-B dzak et al. (2016) found positive associations between CRP and IL-18, between CRP and ICAM-1, and between IL-18 and ICAM-1 in serum from 81 ankylosing spondylitis patients. CRP, IL-18, and ICAM-1 all loaded onto blood factor 1 in our study. In an *in vivo* human tissue study of airway epithelium regeneration, Coraux et al. (2005) found IL-8 increased during the initial stages of airway epithelium regeneration followed by an increase of MMP-7 in later stages. Both MMP-7 and IL-8 loaded onto blood factor 5 in our study. In a study of 21 rats, Kocael et al. (2016) found tissue levels of MMP-9 were positively correlated with tissue levels of MPO. Both MMP-9 and MPO loaded onto blood factor 8 in our study. Finally, in a study of zebrafish, Chen et al. (2016) found increased IL-1 β in the spleen and increased SOD in the liver after exposure to graphene oxide, which is a nanomaterial. Both IL-1 β and SOD loaded onto blood factor 9 in our study.

It is worth noting that demographic, lifestyle, medical, and occupational characteristics are associated with changes in various biomarkers. Although we adjusted for these characteristics in our analysis, we derived the factors before estimating adjusted associations between CNT/F exposure and the factors, so perhaps this contributed to our inability to provide more complete interpretations or explanations of the factors and their associations with CNT/F exposure.

The workers in our study had a median background-corrected inhalable elemental carbon concentration of 0.24 $\mu\text{g}/\text{m}^3$ and a median background-corrected respirable elemental carbon concentration of 0.096 $\mu\text{g}/\text{m}^3$ (Schubauer-Berigan et al., unpublished results). NIOSH has established a recommended exposure limit (REL) for CNT/F exposure of an eight-hour time weighted average of 1 $\mu\text{g}/\text{m}^3$ of background corrected elemental carbon, a marker of CNT/F, at the respirable fraction (National Institute for Occupational Safety and Health, 2013). The REL, based on evidence from animal studies, was determined in reference to potential for development of pulmonary fibrosis after CNT/F exposure (National Institute for Occupational Safety and Health, 2013). The risk of developing lung effects was estimated at 0.5–16% over a working lifetime (National Institute for Occupational Safety and Health,

2013). With median background-corrected respirable exposure levels of elemental carbon well below the NIOSH REL (fewer than 10 workers had background-corrected respirable EC >1 $\mu\text{g}/\text{m}^3$) and median (interquartile range) duration of CNT/F exposure in current and past jobs of 3.89 (4.67) years, overt outcomes were not expected (Erdely et al., 2013, National Institute for Occupational Safety and Health, 2013).

Only one human epidemiological study of sputum biomarkers in CNT/F workers has been conducted. A cross-sectional study at one site in Russia found increased sputum levels of MUC-1/KL-6, interleukin-1 beta (IL-1 β), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), IL-8, and TNF- α , but not of interleukin-2 (IL-2), among 10 workers exposed to MWCNT compared to 12 unexposed workers (Fatkhutdinova et al., 2016). Although not a study of sputum, another cross-sectional study at one site in Korea found higher levels of oxidative stress biomarkers in exhaled breath condensate of nine MWCNT manufacturing workers compared to those of four office workers (Lee et al., 2015). In our study, at least one CNT/F metric was associated with sputum IL-8 (inverse) and TNF- α (inverse, positive), but we did not find associations between CNT/F and sputum MUC-1/KL-6, IL-1 β , IL-2, IL-4, IL-5, or IL-6. We also observed associations between at least two CNT/F metrics and 8-OHdG (positive), GPx (inverse, positive), MPO (inverse), and SOD (positive). Several factors could potentially explain discrepancies between our results and those of Fatkhutdinova et al. (2016). The study by Fatkhutdinova et al. (2016) included 22 workers at a single MWCNT-producing site, the study was conducted in Russia meaning important genetic, ethnic, social, economic, dietary, or other factors likely differed, the workers in the study by Fatkhutdinova et al. (2016) had higher CNT exposures (mean inhalable EC: 8.5 $\mu\text{g}/\text{m}^3$, mean respirable EC: 1.6 $\mu\text{g}/\text{m}^3$), and we were able to adjust for more potential confounders in our analysis because of our larger sample size. In addition, the sputum we collected in our study may have represented upper respiratory tract or oral cavity secretions while the quality of sputum from the study by Fatkhutdinova et al. (2016) is unknown.

A few human epidemiological studies of relationships between CNT/F exposure and blood biomarkers have been conducted (Table 6). In reference to the analytes measured in our study, a cross-sectional study at 14 sites in Taiwan found decreased levels of SOD and increased levels of fibrinogen and ICAM-1 in blood of 227 workers exposed to engineered carbonaceous nanomaterials, including 52 workers exposed to CNT, relative to those of 137 unexposed workers (Liou et al., 2012). The same study also found no difference in blood levels of 8-OHdG, GPx, MPO, VCAM, and IL-6 between exposed and unexposed workers. A six-month follow-up exam of 124 workers exposed to engineered carbonaceous nanomaterials and 77 unexposed workers from the same 14 sites in Taiwan found blood levels of SOD and GPx had greater decreases from baseline levels among the exposed workers than among the unexposed workers (Liao et al., 2014). Blood levels of VCAM had greater increases from baseline levels among the exposed than unexposed workers, but changes from baseline blood levels of 8-OHdG, MPO, fibrinogen, ICAM, and IL-6 did not differ among exposed and unexposed workers. When the exposed group was restricted to workers who were exposed to CNT, only the GPx finding remained significant. Increased serum levels of IL-1 β , IL-4, interleukin-10 (IL-10), and TNF- α , but not of MUC-1/KL-6, SPP-1, IL-2, IL-5, or IL-8, were measured among exposed workers relative to unexposed

workers (Fatkhutdinova et al., 2016). A cross-sectional study at one site in Europe found increased blood levels of ICAM-1, but not of CRP, eotaxin, fibrinogen, IL-6, IL-8, MDC, t-PA, TNF- α , VCAM-1, or vWF, among 22 workers exposed to MWCNT compared to 39 or 42 unexposed workers (the number of unexposed workers varied by biomarker) (Kuijpers et al., 2018; Vlaanderen et al., 2017). A four-month follow-up exam of 10 or 13 exposed workers, depending on the biomarker, and 6 unexposed workers from the same site in Europe found increased blood levels of ICAM-1, IL-8, and MDC, but not of CRP, eotaxin, fibrinogen, IL-6, t-PA, TNF- α , VCAM-1, or vWF, among exposed workers compared to unexposed workers. In our study, at least one CNT/F metric was associated with blood eotaxin-1 (inverse), IL-8 (inverse), 8-OHdG (inverse, positive), GPx (inverse, positive), SOD (inverse, positive), fibrinogen (inverse, positive), ICAM-1 (positive), t-PA (inverse), VCAM-1 (positive), and vWF (inverse) and at least one direct-reading instrument metric was associated with blood MUC-1/KL-6 (positive), CRP (positive), and MPO (positive). We did not find associations between CNT/F or direct-reading instrument metrics and blood SPP-1, IL-1 β , IL-10, or MDC and we were unable to analyze IL-2, IL-4, IL-5, IL-6, and TNF- α because too many values were measured below detection limits.

There was no consistency of the altered markers in the sputum and circulation. Although reasons for the differences between results of analyses based on sputum and blood factors and biomarkers are unknown, they are perhaps due to sputum biomarkers being associated more with the site of potential deposition and, therefore, more reflective of the initial pulmonary response to CNT/F exposure, whereas blood biomarkers are more reflective of secondary responses. This conjecture may also be supported by our observation of primarily structural remodeling and oxidative stress proteins in sputum, with a more diffuse response in blood. The reliability of these measures was also confounded by the generally low occupational exposures.

Our results support consideration of occupational exposure limits (OEL) based on additional/alternative exposure metrics because we observed associations between inhalable EC and CNT/F structure counts and sputum and blood biomarkers that we did not observe for respirable EC. NIOSH acknowledged that alternative exposure metrics, in addition to respirable EC, may be useful for the development of OEL for CNT/F exposure (National Institute for Occupational Safety and Health, 2013). For example in the sputum, several analytes (MMP-2, SPP-1, TIMP-1, 8-OHdG, fibrinogen, VCAM-1) were associated with inhalable EC and not respirable EC. A recent animal study found that inhalable CNT/F deposits primarily in the conducting airways and can cause proliferative bronchiolitis obliterans (Bishop et al., 2017). In the analysis of circulating mediators, CNT/F structure counts were associated with changes in mediators such as increased MMP-9, TIMP-1, EDN1, ICAM-1, VCAM-1 and decreased SOD and t-PA which was not observed for either inhalable or respirable EC. All of these changes suggest systemic inflammation and potential for cardiovascular dysfunction following a pulmonary exposure (Aragon et al., 2016; Erdely et al., 2009, 2011; Gupta et al., 2009; Lund et al., 2009). Our results, and those of Bishop et al. (2017), suggest that additional/alternative exposure metrics, such as inhalable EC and total CNT/F structures, and health outcomes, such as cancer, cardiovascular diseases, other respiratory diseases, or other outcomes related to oxidative stress and, to a lesser extent, inflammation, should be considered when establishing an OEL for CNT/F.

Results for direct-reading instruments did not consistently follow the pattern of results observed for CNT/F exposure metrics. MMP-7 and MMP-9 in sputum and factor 2, MUC-1/KL-6, MMP-7, CRP, IL-6R- β , MPO, and PAI-1 in blood were related only to a direct-reading instrument metric(s) in our study. Perhaps this is not surprising given the lack of associations between direct-reading instrument measurements and filter-based measurements of EC and CNT/F structures we noted previously (Dahm et al., 2013). The direct-reading instrument metrics indicated exposure to general ultrafine particulates (i.e., not confined to CNT/F). Therefore, it is presumed likely that associations we observed between direct-reading instrument metrics and factors or biomarkers reflect associations with exposure from other indoor/outdoor sources of particulates (e.g., related to combustion processes, polymer dust, or air pollution infiltration) or with a combined exposure to airborne particulates and CNT/F.

Workers in our study were likely exposed to a wider variety of CNT/F types and catalysts, including metals, than workers in previous studies. This may have concealed associations between exposure to specific types of CNT/F (e.g., single-walled CNT [SWCNT] vs. MWCNT vs. CNF) and biomarkers of early effect. We were unable to take into consideration CNT/F type because, as shown in Table 1 of Dahm et al. (2018), many workers who were exposed to SWCNT were also exposed to MWCNT. Similarly, workers who were exposed to CNF were also exposed to MWCNT. Furthermore, our PBZ EC and CNT/F in sputum exposure metrics were unable to distinguish between SWCNT, MWCNT, and CNF and our exposure metrics based on PBZ CNT/F structures identified by TEM were not analyzed in a quantitative way (e.g., number of SWCNT vs. number of MWCNT vs. number of CNF). Finally, we observed a large amount of heterogeneity in diameter and length among the MWCNT types that were used by workers in the study, which further complicated consideration of CNT type.

The strengths of our study include its large sample size relative to previous studies, industrywide representation of CNT/F activities, personally monitored exposure, variety of exposure metrics (including an internal exposure metric: CNT/F in sputum), use of factor analysis to identify biomarkers response patterns, and adjustment for several potential confounders.

Limitations of our study include its cross-sectional design, lack of sputum samples for 17% of enrolled workers, and reduced power and precision of estimates due to lower CNT/F exposure levels than previous studies. The amount of biomarker values measured below detection limits also reduced the number of biomarkers we could include in the factor analysis and evaluate in relation to CNT/F exposure. Residual confounding is possible because we did not have a larger sample size which would have enabled us to more completely adjust for potential confounders incorporated into the CHM (e.g., body mass index, waist circumference, cigarette smoking). Although multiple comparisons adjustments are not recommended for our study because it is not based on a random sample, does not include random exposure assignment, and assignment of exposures cannot be thought of as approximately random (Greenland and Robins, 1991), some of the associations we observed may be false positives given the large number of associations we evaluated. Finally, the quality of almost all sputum samples was poor, and the specimens we collected may have

reflected upper respiratory tract secretions. This would be consistent with the lack of associations between sputum biomarkers and respirable EC that we observed. With respect to CNT/F exposure, this may have resulted in non-differential misclassification of sputum biomarker levels and, therefore, may have biased our estimates of association toward the null.

5. Conclusions

In conclusion, we observed several associations between CNT/F exposure and sputum and blood biomarkers of early effect in this industrywide cross-sectional epidemiological study of U.S. workers. Although these results require replication in other exposed populations, they provide evidence for the establishment of additional OEL(s) that consider alternative CNT/F exposure metrics and health outcomes. They also provide impetus for future prospective cohort studies of CNT/F workers to establish temporal relationships with biomarker and overt health outcomes, such as cancer, cardiovascular and respiratory diseases, and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

8-OHdG	8-hydroxy-2'-deoxyguanosine
AIC	Akaike Information Criterion
α-2-M	Alpha-2-Macroglobulin
Apo-AI	Apolipoprotein A-I
Apo-AII	Apolipoprotein A-II
CRP	C-reactive protein
CNF	carbon nanofibers
CNT	carbon nanotubes

CNT/F	carbon nanotubes and carbon nanofibers
CHM	cardiovascular health metric
C3	Complement C3
CPC	condensation particle counter
CI	confidence interval
ELPI	electrical low-pressure impactor
EC	elemental carbon
ECN	engineered carbonaceous nanomaterials
GPx	Glutathione peroxidase
GM-CSF	Granulocyte-macrophage colony-stimulating factor
ICAM-1	Intercellular adhesion molecule 1
IL-1 β	Interleukin-1 beta
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-6R-β	Interleukin-6 receptor subunit beta
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12p70	Interleukin-12 subunit p70
IL-18	Interleukin-18
IARC	International Agency for Research on Cancer
IQR	interquartile range
LDD	least detectable dose
MDC	C-C motif chemokine 22/Macrophage derived chemokine
MMP-1	Interstitial collagenase/Matrix metalloproteinase-1
MMP-2	72 kDa type IV collagenase/Matrix metalloproteinase-2
MMP-7	Matrilysin/Matrix metalloproteinase-7
MMP-9	Matrix metalloproteinase-9

MUC-1/KL-6	Mucin-1/Krebs von den Lungen-6
MWCNT	multi-walled carbon nanotubes
MPO	Myeloperoxidase
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NMAM	NIOSH Manual of Analytical Methods
Ln	natural logarithm of linear
NSAID	non-steroidal anti-inflammatory drugs
OEL	occupational exposure limits
PM_{2.5}	particulate matter mass with an aerodynamic diameter ≤ 2.5 μm
PBZ	personal breathing zone
DustTrak	photometer/optical particle counter
PAI-1	Plasminogen activator inhibitor 1
EDN1	Endothelin-1
REL	recommended exposure limit
SPP-1	Osteopontin/Secreted phosphoprotein 1
SWCNT	single-walled carbon nanotubes
SOD	Superoxide dismutase
TIMP-1	Metalloproteinase inhibitor 1/Tissue inhibitor of metalloproteinases 1
t-PA	Tissue-type plasminogen activator
TEM	transmission electron microscopy
TNF-α	Tumor necrosis factor alpha
U.S	United States
U.S. EPA	U.S. Environmental Protection Agency
VCAM-1	Vascular cell adhesion protein 1
vWF	von Willebrand factor

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Highlights

- We evaluated carbon nanotube/nanofiber (CNT/F) exposure in relation to biomarkers.
- We assessed CNT/F exposure via personal breathing zone, filter-based air sampling.
- We measured fibrosis, inflammation, oxidative stress, cardiovascular biomarkers.
- Inhalable rather than respirable CNT/F was more often associated with biomarkers.
- Sixteen biomarkers were associated with at least three CNT/F exposure metrics.

Figure 1. Flow diagram depicting companies and workers that participated in a cross-sectional epidemiological study of CNT/F workers in the U.S. Solid boxes or lines represent companies and/or workers who advanced past each step shown; dashed boxes or lines represent companies and/or workers excluded after each step shown (see Sections 2.1 and 2.2 for more details). Abbreviations: CNT/F, carbon nanotubes and carbon nanofibers; ECN, engineered carbonaceous nanomaterials; U.S. United States.

Table 1

Descriptive statistics for demographic, lifestyle, medical, and occupational characteristics of CNT/F workers, United States, 2012–2014.

Characteristic	n (%)
Total ^a	103 (100)
Age at interview/exam (years)	
20–30	21 (20)
31–40	26 (25)
41–50	18 (17)
51–60	30 (29)
60–67	8 (8)
Median (IQR)	44.61 (22.76)
Sex	
Male	83 (81)
Female	20 (19)
Race/ethnicity	
Non-Hispanic White	84 (82)
Asian	9 (9)
Other or Multiple	10 (10)
Level of schooling	
High school graduate	10 (10)
Vocational school or some college	24 (23)
College graduate	29 (28)
Postgraduate	40 (39)
Body mass index (kg/m ²)	
19–<25	35 (34)
25–<30	49 (48)
30–<35	10 (10)
35–47	9 (9)
Median (IQR)	26.49 (4.96)
Waist circumference (cm)	
69–88	39 (38)

Characteristic	n (%)
>88–102	41 (40)
>102–149	23 (22)
Median (IQR)	92.20 (17.80)
Ever smoke cigarettes (at least 100 lifetime)	
Never	64 (62)
Past	24 (23)
Current	15 (15)
Total pack-years smoked cigarettes, median (IQR) ^b	10.50 (20.39)
Ever drink alcoholic beverages at least 12 times in a single year	
Never or past	35 (34)
Current	68 (66)
Total years drank at least 12 alcoholic beverages in a single year, median (IQR) ^c	15.00 (23.00)
During years drank alcohol, usual number of drinks ^d per week, median (IQR) ^c	4.00 (4.50)
Currently have a cold or other upper airway infectious disease	
No	94 (91)
Yes	9 (9)
Ever had asthma	
Never	85 (83)
Past	10 (10)
Current	8 (8)
Doctor ever told you that you had heart trouble	
No	93 (90)
Yes	10 (10)
Doctor ever told you that you have high blood pressure	
No	73 (72)
Yes	29 (28)
Missing	1
Ever had diabetes	
No	97 (94)
Yes	6 (6)
Currently taking any prescription or non-prescription non-steroidal anti-inflammatory drugs	

Characteristic	n (%)
No	76 (74)
Yes	27 (26)
Currently taking any prescription or non-prescription cholesterol medication	
No	89 (86)
Yes	14 (14)
Currently taking any prescription or non-prescription hypertension medication	
No	87 (84)
Yes	16 (16)
Cardiovascular health metric score ^e	
0–2	11 (11)
3	11 (11)
4	26 (25)
5	30 (29)
6	25 (24)
Regular work shift	
Days	97 (94)
Other	6 (6)
Hours per week usually work, median (IQR)	40.00 (5.00)

Abbreviations: CNF, carbon nanofiber(s); CNT, carbon nanotube(s); IQR, interquartile range.

^aFive individuals did not provide a sputum or blood sample, so they were excluded from the analysis.

^bFor the 39 past or current cigarette smokers only.

^cFor the 97 past or current alcohol drinkers only.

^dA “drink” was defined as “cans or glasses of beer, shots of hard liquor straight or in a mixed drink”.

^eOne point each scored for body mass index $<25 \text{ kg/m}^2$, waist circumference $<102 \text{ cm}$ (men) or $<88 \text{ cm}$ (women), not a current cigarette smoker (at least 100 lifetime), not ever told by a doctor that you have high blood pressure, not currently taking any prescription or non-prescription cholesterol medication, and not ever having diabetes.

Table 2

Factor loadings^a for sputum biomarkers of effect^b for 90 CNT/F workers^c, United States, 2012–2014.

Biomarker ^{d, e}	Factor ^{f, g}		
	1	2	3
<i>Cancer/Fibrosis</i>			
MUC-1/KL-6	0.16	0.84	0.08
MMP-2	0.77	0.44	0.15
MMP-7	0.09	0.88	0.16
TIMP-1	0.65	0.54	0.25
<i>Inflammation</i>			
α-2-M	0.93	0.07	0.07
Apo-AI	0.93	0.18	0.16
Apo-AII	0.91	0.24	0.02
IL-1 β	0.66	0.28	0.55
IL-6R-β ^h	0.43	0.65	0.27
IL-8	0.59	0.66	0.31
IL-18 ^h	0.07	0.19	0.88
<i>Oxidative Stress</i>			
8-OHdG	0.45	0.38	−0.36
MPO	0.48	0.31	0.69
<i>Cardiovascular/Coagulation</i>			
Fibrinogen	0.71	0.23	0.36
PAI-1 ^h	0.58	0.53	0.27
VCAM-1 ^h	0.87	0.28	0.19

Abbreviations: CNF, carbon nanofiber; CNT, carbon nanotube.

^aLoadings in bold have the greatest magnitude for the respective biomarkers. These loadings were used to name the factors.

^bThe following 20 sputum biomarkers of effect were not included in the factor analysis due to the decision rules shown in Table S4 (i.e., none of the 20 sputum biomarkers had >90% of values above the least detectable dose): MMP-1, MMP-9, SPP-1, CRP, eotaxin-1, GM-CSF, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, MDC, TNF-α, GPx, SOD, EDN1, ICAM-1, t-PA, and vWF.

^cEighteen workers did not provide sputum samples, so they were excluded from the analysis.

^dSee Table S1 for definitions of biomarker abbreviations.

^eTransformed by taking the natural logarithm of the original values (i.e., ln(x)).

^fThe eigenvalues for factors 1–3 were 9.57, 1.67, and 1.28, respectively.

^gThe percentage of variation in the data explained by factors 1–3 was 59.80, 10.46, and 8.02, respectively.

^hOne value for IL-6R-β, two for IL-18, one for PAI-1, and one for VCAM-1 were imputed as one-half the least detectable dose.

Associations between CNT/F and direct-reading instrument exposure variables and sputum biomarker factors among 90 workers^a, United States, 2012–2014.

Table 3

Variable ^b	Factor		
	1	2	3
Inhalable EC			
Linear ^f	0.0042 (0.000038, 0.0084)	0.004 (−0.00061, 0.0086)	0.015 (−0.055, 0.085)
Ln			
Respirable EC			
Linear ^f	0.024 (−0.011, 0.059)	0.024 (−0.014, 0.063)	
Ln			0.071 (−0.026, 0.17)
Total CNT			
Linear ^f		−0.17 (−0.57, 0.23)	
Ln	0.041 (−0.034, 0.12)		−0.088 (−0.17, −0.0085)
Single CNT			
Linear ^f		−0.49 (−1.5, 0.48)	0.48 (−0.47, 1.4)
Ln	0.043 (−0.021, 0.11)		
CNT <1 μm			
Linear ^f		−0.61 (−1.6, 0.36)	
Ln	0.023 (−0.033, 0.078)		−0.042 (−0.1, 0.018)
CNT <2 μm			
Linear ^f		−0.55 (−1.5, 0.39)	
Ln	0.016 (−0.039, 0.07)		−0.055 (−0.11, 0.0022)
CNT <5 μm			
Linear ^f	0.11 (−0.44, 0.66)		
Ln		−0.029 (−0.088, 0.031)	−0.072 (−0.13, −0.015)
CNT <10 μm			
Linear ^f			

Variable ^b	Factor		
	1	2	3
Ln	Adjusted ^c β (95% CI) ^{d, e} 0.027 (−0.03, 0.084)	Adjusted ^c β (95% CI) ^{d, e} −0.027 (−0.089, 0.035)	Adjusted ^c β (95% CI) ^{d, e} −0.07 (−0.13, −0.011)
CPC			
Linear ^f		−4E-6 (−0.000027, 0.000019)	−4.2E-6 (−0.000026, 0.000018)
Ln	0.088 (−0.13, 0.3)		
ELPI			
Linear ^f	−3.1E-6 (−0.000026, 0.00002)	0.000014 (−0.000012, 0.000039)	8.6E-6 (−0.000017, 0.000034)
Ln			
PM _{2.5}			
Linear ^f	−0.014 (−0.039, 0.012)	−0.011 (−0.04, 0.017)	
Ln			0.007 (−0.22, 0.23)
Sputum CNT			
No	Reference	Reference	Reference
Yes	−0.16 (−0.64, 0.33)	0.28 (−0.25, 0.81)	−0.22 (−0.75, 0.3)
Job title			
Administrator	0.52 (−0.13, 1.2)	0.071 (−0.64, 0.78)	−0.39 (−1.1, 0.31)
Chemist	−0.27 (−1.2, 0.68)	−0.94 (−2, 0.095)	−0.7 (−1.7, 0.33)
Engineer or maintenance	0.34 (−0.29, 0.96)	−0.31 (−0.99, 0.38)	−0.027 (−0.71, 0.65)
Research and development	0.47 (−0.2, 1.1)	−0.055 (−0.78, 0.67)	0.2 (−0.52, 0.92)
Technician	Reference	Reference	Reference
Any CNT ^g			
No	Reference	Reference	Reference
Yes	0.21 (−0.4, 0.82)	−0.053 (−0.72, 0.61)	−0.098 (−0.76, 0.56)
CNT duration			
Linear ^f	0.02 (−0.026, 0.067)	0.026 (−0.026, 0.077)	0.014 (−0.037, 0.065)
Ln			

Abbreviations: AIC, Akaike Information Criterion; CI, confidence interval; CNF, carbon nanofibers; CNT, carbon nanotubes; Ln, natural logarithm of linear.

^aEighteen workers did not provide sputum samples, so they were excluded from the analysis.

^qSee Table S3 for definitions of exposure variable abbreviations.

^cAdjusted for age at interview/exam (continuous linear term in unrounded years), sex, race/ethnicity (non-Hispanic White, other), level of schooling (high school graduate, vocational school, or some college; college graduate or postgraduate), and cardiovascular metric health score (0–4, 5–6).

^dEstimated via linear regression models.

^e β (95% CI) in b old have p-values <0.05.

^fThe β (95% CI) for a continuous linear term is shown only if the continuous linear term had a lower AIC value than the natural logarithm of a continuous linear term and vice versa.

^gDetermined via transmission electron microscopy.

Table 4

Factor loadings^a for blood biomarkers of effect^b for 102 CNT/F workers^c, United States, 2012–2014.

Biomarker ^{d, e}	Factor ^{f, g}								
	1	2	3	4	5	6	7	8	9
<i>Cancer/Fibrosis</i>									
MUC-1/KL-6	-0.04	0.20	-0.77	-0.02	0.04	0.05	0.18	-0.09	-0.03
MMP-2	-0.29	0.29	-0.10	0.27	-0.12	0.20	0.45	0.40	0.00
MMP-7	0.07	0.27	-0.19	-0.10	0.70	0.06	0.24	0.11	-0.13
MMP-9	0.14	-0.02	-0.03	0.03	0.53	-0.04	-0.06	0.54	0.18
SPP-1	-0.08	-0.07	0.00	0.61	-0.11	-0.05	-0.16	0.24	0.37
TIMP-1	0.20	0.30	0.02	0.56	0.20	0.32	0.12	-0.15	-0.22
<i>Inflammation</i>									
α-2-M	-0.09	-0.54	-0.38	-0.12	0.03	-0.02	0.23	0.09	0.48
Apo-AI	-0.07	-0.24	0.17	0.04	0.19	0.11	0.75	-0.20	0.26
Apo-AII	-0.01	0.28	0.75	0.12	-0.02	0.13	0.13	-0.30	0.02
CRP ^h	0.73	0.12	0.21	-0.15	0.03	0.34	-0.01	-0.06	-0.15
C3	0.65	0.14	0.25	-0.08	0.19	0.16	0.10	0.19	0.07
IL-1β ^h	0.32	-0.02	-0.15	-0.01	0.37	0.00	0.29	-0.08	0.38
IL-6R-β	0.11	0.29	0.19	0.60	0.00	-0.28	0.24	0.09	0.15
IL-8 ^h	0.23	0.13	0.04	0.07	0.71	0.14	-0.04	0.01	0.02
IL-18	0.62	0.27	0.00	0.27	0.03	-0.15	-0.02	-0.03	0.00
MDC	-0.08	0.59	0.10	-0.12	0.21	0.18	-0.27	-0.01	0.23
<i>Oxidative Stress</i>									
8-OHdG	0.05	0.09	0.75	0.08	-0.05	0.19	-0.01	-0.02	-0.18
GPx	0.14	0.01	-0.27	-0.22	-0.03	-0.17	0.63	0.17	-0.03
MPO	-0.06	0.06	-0.08	0.24	0.05	-0.03	0.03	0.82	-0.13
SOD ^h	-0.07	0.13	-0.04	0.03	-0.02	0.02	0.07	-0.06	0.78
<i>Cardiovascular/Coagulation</i>									
EDN1	-0.09	0.02	0.06	0.45	0.42	-0.18	-0.28	-0.04	-0.01
Fibrinogen	0.46	0.09	0.08	0.00	0.14	0.71	0.17	0.26	0.10

Biomarker ^{d, e}	Factor ^{f, g}								
	1	2	3	4	5	6	7	8	9
ICAM-1 ^h	0.65	0.03	-0.20	0.11	0.15	-0.11	-0.03	-0.09	-0.06
PAI-1	0.25	0.72	0.07	0.13	0.19	0.11	0.13	0.20	0.08
t-PA	0.29	0.76	-0.09	0.03	0.08	0.10	-0.03	-0.04	-0.03
VCAM-1	0.09	-0.09	0.09	0.75	-0.01	0.17	-0.08	0.19	-0.14
vWF	-0.12	0.27	0.22	0.06	0.05	0.79	-0.12	-0.17	-0.01

Abbreviations: CNF, carbon nanofiber; CNT, carbon nanotube; C3, complement C3.

^aLoadings in bold have the greatest magnitude for the respective biomarkers. These loadings were used to name the factors.

^bThe following 10 blood biomarkers of effect were not included in the factor analysis due to the decision rules shown in Table S4 (i.e., none of the 10 blood biomarkers had >90% of values above the least detectable dose): MMP-1, eotaxin-1, GM-CSF, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, and TNF-α.

^cSix workers did not provide blood samples, so they were excluded from the analysis.

^dSee Table S1 for definitions of biomarker abbreviations.

^eTransformed by taking the natural logarithm of the original values (i.e., ln(x)).

^fThe eigenvalues for factors 1–9 were 4.54, 2.93, 2.36, 2.36, 1.79, 1.64, 1.38, 1.32, 1.05, and 1.01, respectively.

^gThe percentage of variation in the data explained by factors 1–9 was 16.80, 10.86, 8.75, 6.64, 6.06, 5.10, 4.89, 3.89, and 3.75, respectively.

^hOne value for CRP, one for IL-1 β, seven for IL-8, eight for SOD, and one for ICAM-1 were imputed as one-half the least detectable dose.

Associations between CNT/F and direct-reading instrument exposure variables and blood biomarker factors among 102 workers^a, United States, 2012–2014.

Table 5

Variable ^b	Factor								
	1	2	3	4	5	6	7	8	9
Inhalable EC									
Linear ^f				−0.002 (−0.0063, 0.0023)		−0.006 (−0.01, −0.0017)			−0.0069 (−0.011, −0.0026)
Ln	0.024 (−0.036, 0.083)	0.031 (−0.025, 0.086)	0.039 (−0.024, 0.1)		0.057 (−0.0058, 0.12)		0.0093 (−0.048, 0.067)	0.035 (−0.03, 0.099)	
Respirable EC									
Linear ^f					0.019 (−0.019, 0.057)	−0.063 (−0.098, −0.027)			−0.07 (−0.11, −0.035)
Ln	0.033 (−0.051, 0.12)	−0.038 (−0.12, 0.04)	−0.11 (−0.2, −0.022)	−0.036 (−0.12, 0.048)			0.08 (−0.00074, 0.16)	0.075 (−0.016, 0.17)	
Total CNT									
Linear ^f						−0.6 (−0.98, −0.23)			−0.84 (−1.2, −0.49)
Ln	0.093 (0.027, 0.16)	−0.022 (−0.086, 0.042)	0.081 (0.01, 0.15)	0.057 (−0.011, 0.12)	0.087 (0.016, 0.16)			0.037 (−0.038, 0.11)	
Single CNT									
Linear ^f	−0.27 (−1.2, 0.63)			0.078 (−0.83, 0.98)		−1.5 (−2.4, −0.6)		−0.21 (−1.2, 0.77)	−2 (−2.8, −1.1)
Ln		0.024 (−0.035, 0.083)	0.043 (−0.024, 0.11)		0.012 (−0.056, 0.08)		0.035 (−0.026, 0.096)		
CNT <1 μm									
Linear ^f	−0.28 (−1.2, 0.62)			0.046 (−0.86, 0.95)		−1.6 (−2.5, −0.7)			−1.9 (−2.8, −1)
Ln		−0.015 (−0.065, 0.034)	−0.023 (−0.079, 0.033)		0.049 (−0.0071, 0.1)			0.022 (−0.035, 0.079)	
CNT <2 μm									
Linear ^f		−0.25 (−1.1, 0.56)				−1.6 (−2.4, −0.69)			−1.8 (−2.7, −0.99)

Variable ^b	Factor								
	1	2	3	4	5	6	7	8	9
Ln	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}	Adjusted ^c β (95% CI) ^{d, e}
CNT <5 μ m	0.024 (-0.028, 0.076)		0.019 (-0.036, 0.075)	0.014 (-0.038, 0.066)	0.05 (-0.0051, 0.1)		0.027 (-0.023, 0.077)	0.027 (-0.03, 0.083)	
Linear ^f						-0.94 (-1.5, -0.39)	0.31 (-0.22, 0.84)		-1.2 (-1.8, -0.72)
Ln	0.061 (0.01, 0.11)	-0.016 (-0.065, 0.032)	0.05 (-0.0047, 0.1)	0.034 (-0.017, 0.086)	0.075 (0.021, 0.13)			0.024 (-0.032, 0.081)	
CNT <10 μ m									
Linear ^f						-0.65 (-1, -0.25)	0.21 (-0.17, 0.59)		-0.9 (-1.3, -0.52)
Ln	0.069 (0.017, 0.12)	-0.024 (-0.074, 0.026)	0.062 (0.0061, 0.12)	0.04 (-0.013, 0.094)	0.077 (0.021, 0.13)			0.021 (-0.037, 0.079)	
CPC									
Linear ^f			-0.000016 (-0.000028, -4.7E-6)	3.3E-6 (-8E-6, 0.000015)	4.6E-6 (-7.5E-6, 0.000017)	-1.3E-6 (-0.000013, 0.000011)			-6.3E-6 (-0.000018, 5.5E-6)
Ln	-0.093 (-0.29, 0.1)	0.14 (-0.042, 0.32)							
ELPI									
Linear ^f	0.000015 (1.8E-6, 0.000028)		-0.28 (-0.45, -0.096)	2.3E-6 (-0.000011, 0.000016)		-1.1E-6 (-0.000015, 0.000013)			-0.000012 (-0.000026, 1.8E-6)
Ln		0.11 (-0.055, 0.27)			-0.066 (-0.25, 0.12)		0.23 (0.068, 0.4)	0.17 (-0.019, 0.36)	
PM _{2.5}									
Linear ^f			-0.0048 (-0.026, 0.017)	-0.074 (-0.26, 0.12)		0.0078 (-0.014, 0.029)			
Ln	-0.15 (-0.34, 0.04)	0.26 (0.085, 0.43)			-0.1 (-0.3, 0.1)		0.34 (0.16, 0.51)	0.26 (0.061, 0.46)	-0.11 (-0.31, 0.093)
Sputum CNT									
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.073 (-0.43, 0.57)	0.12 (-0.35, 0.58)	0.34 (-0.19, 0.86)	0.2 (-0.24, 0.65)	0.34 (-0.21, 0.88)	-0.34 (-0.85, 0.17)	0.00075 (-0.49, 0.49)	-0.22 (-0.75, 0.3)	0.29 (-0.21, 0.8)
Job title									

Variable ^b	Factor								
	1	2	3	4	5	6	7	8	9
Administrator	Adjusted ^c β (95% CI) ^{d, e} 0.18 (-0.43, 0.78)	Adjusted ^c β (95% CI) ^{d, e} -0.061 (-0.62, 0.5)	Adjusted ^c β (95% CI) ^{d, e} 0.58 (-0.038, 1.2)	Adjusted ^c β (95% CI) ^{d, e} 0.26 (-0.33, 0.85)	Adjusted ^c β (95% CI) ^{d, e} -0.39 (-1, 0.23)	Adjusted ^c β (95% CI) ^{d, e} 0.29 (-0.34, 0.91)	Adjusted ^c β (95% CI) ^{d, e} -0.11 (-0.68, 0.46)	Adjusted ^c β (95% CI) ^{d, e} -0.35 (-0.98, 0.27)	Adjusted ^c β (95% CI) ^{d, e} -0.18 (-0.8, 0.44)
Chemist	-0.05 (-1, 0.9)	0.14 (-0.74, 1)	0.67 (-0.31, 1.6)	-0.32 (-1.3, 0.62)	0.61 (-0.37, 1.6)	-0.055 (-1, 0.93)	0.52 (-0.38, 1.4)	0.61 (-0.38, 1.6)	-0.36 (-1.3, 0.62)
Engineer or maintenance	0.15 (-0.42, 0.72)	0.15 (-0.38, 0.68)	0.23 (-0.36, 0.81)	-0.064 (-0.63, 0.5)	-0.14 (-0.73, 0.45)	-0.17 (-0.76, 0.43)	0.23 (-0.31, 0.77)	0.37 (-0.23, 0.96)	-0.059 (-0.65, 0.53)
Research and development	0.21 (-0.45, 0.86)	0.29 (-0.32, 0.9)	-0.13 (-0.8, 0.55)	-0.24 (-0.89, 0.41)	-0.69 (-1.4, -0.019)	-0.22 (-0.9, 0.46)	0.42 (-0.2, 1)	-0.12 (-0.8, 0.56)	0.43 (-0.24, 1.1)
Technician	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Any CNT ^g	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.59 (0.076, 1.1)	-0.032 (-0.52, 0.46)	0.81 (0.28, 1.3)	0.65 (0.15, 1.2)	0.13 (-0.43, 0.68)	0.32 (-0.22, 0.87)	-0.51 (-1, -0.015)	-0.037 (-0.6, 0.53)	-0.23 (-0.78, 0.32)
CNT duration									
Linear ^f		-0.014 (-0.055, 0.028)	-0.03 (-0.094, 0.033)	-0.021 (-0.065, 0.024)	-0.0013 (-0.049, 0.046)	-0.038 (-0.085, 0.0078)	-0.011 (-0.069, 0.047)	-0.0057 (-0.054, 0.043)	-0.058 (-0.1, -0.013)
Ln	0.042 (-0.017, 0.1)								

Abbreviations: AIC, Akaike Information Criterion; CI, confidence interval; CNF, carbon nanofibers; CNT, carbon nanotubes; Ln, natural logarithm of linear.

^aSix workers did not provide blood samples, so they were excluded from the analysis.

^bSee Table S3 for definitions of exposure variable abbreviations.

^cAdjusted for age at interview/exam (continuous linear term in unrounded years), sex, race/ethnicity (non-Hispanic White, other), level of schooling (high school graduate, vocational school, or some college; college graduate or postgraduate), and cardiovascular metric health score (0-4, 5-6).

^dEstimated via linear regression models.

^e β (95% CI) in bold have p-values <0.05.

^fThe β (95% CI) for a continuous linear term is shown only if the continuous linear term had a lower AIC value than the natural logarithm of a continuous linear term and vice versa.

^gDetermined via transmission electron microscopy.

Table 6

Comparison of associations^a between CNT/F exposure and blood biomarkers of effect that were measured in previous studies and the current study.

Biomarker ^b	Liou et al. (2012)	Liao et al. (2014)	Fatkhutdinova et al. (2016)	Kuijpers et al. (2018); Vlaanderen et al. (2017)		Current study
	Cross-sectional	Longitudinal	Cross-sectional	Cross-sectional	Repeated	Cross-sectional
Cancer/Fibrosis						
MUC-1/KL-6		—	—			↑
SPP-1		—	—			—
Inflammation						
CRP				—	—	↑
Eotaxin-1				—	—	↓
IL-1 β			↑			—
IL-2			—			c
IL-4			↑			c
IL-5			—			c
IL-6	—	—	—	—	—	c
IL-8			—	—	↑	↓
IL-10			↑	—	↑	—
MDC			—	—	↑	—
TNF-α			↑	—	—	c
Oxidative Stress						
8-OHdG	—	—				↓, ↑
GPx	—	↓				↓, ↑
MPO	—	—				↑
SOD	↓	↓				↓, ↑
Cardiovascular/Coagulation						
Fibrinogen	↑	—		—	—	↓, ↑
ICAM-1	↑	—		↑	↑	↑
t-PA				—	—	↓
VCAM-1	—	↑		—	—	↑
vWF				—	—	↓

Abbreviations: CNF, carbon nanofiber; CNT, carbon nanotube.

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↑ indicates an inverse association, ↓ indicates a positive association, and — indicates no association.

^b See Table S1 for definitions of biomarker abbreviations.

^c Excluded from the analysis due to the decision rules shown in Table S4 (i.e., none of these blood biomarkers had >90% of values above the least detectable dose).