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



Matthew M. Dahm^{a,e,*}, Mary K. Schubauer-Berigan^a, Douglas E. Evans^b, M. Eileen Birch^b, Stephen Bertke^a, John D. Beard^{a,c,1}, Aaron Erdely^d, Joseph E. Fernback^b, Robert R. Mercer^d, Sergey A. Grinshpun^e

^a Division of Surveillance, Hazard Evaluations, and Field Studies, National Institute for Occupational Safety and Health, 1090 Tusculum Ave, Cincinnati, OH 45226, USA

^b Division of Applied Research and Technology, National Institute for Occupational Safety and Health, 1090 Tusculum Ave, Cincinnati, OH 45226, USA

^c Epidemic Intelligence Service, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333, USA

^d Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1095 Willowdale Rd, Morgantown, WV 26505, USA

^e Department of Environmental Health, University of Cincinnati, 160 Panzeca Way, Cincinnati, OH 45267, USA

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ABSTRACT

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 $\mu\text{g}/\text{m}^3$ at the respirable size fraction and 6.22 $\mu\text{g}/\text{m}^3$ at the inhalable fraction. Analysis by TEM found a mean exposure of 0.1275 CNT/F structures/ cm^3 , 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.

1. Introduction

The unique strength and conductive properties of carbon nanotubes (CNT) and carbon nanofibers (CNF) have made them attractive for a variety of applications, including composite materials, conductive coatings and films, microelectronics, energy storage devices, and biotechnologies (De Volder et al., 2013). Carbon nanotube and nanofiber (CNT/F) materials and products offer enormous potential to meet growing technological and medical needs, but exposures to CNT/F have also raised early concerns for human and environmental health.

Although health hazards caused by occupational exposures to CNT/F have not been confirmed in humans, accumulating evidence from animal toxicity studies suggests that exposures may cause harm. Studies have indicated that inhalation of certain types of CNT/F may induce localized and systemic inflammation, cytotoxicity, interstitial fibrosis, mutagenesis, and the potential for CNT to promote lung tumorigenesis after inhalation (Lam et al., 2004; Shvedova et al., 2008; Erdely et al., 2009; Ma-Hock et al., 2009; Pauluhn 2010; Porter et al., 2012; Sargent et al., 2014; Kasai et al., 2016).

In a similar fashion, there has been mounting evidence of

* Corresponding author at: Division of Surveillance, Hazard Evaluations, and Field Studies, National Institute for Occupational Safety and Health, 1090 Tusculum Ave, Cincinnati, OH 45226, USA.

E-mail address: mdahm@cdc.gov (M.M. Dahm).

¹ Current address: Brigham Young University, Department of Health Science, 2046 LSB, Provo, UT 84602, USA.

occupational exposures to CNT/F over the past decade. Two recent review articles by [Canu et al. \(2016\)](#) and [Debia et al. \(2016\)](#) identified > 20 published peer-reviewed journal articles or reports that assessed exposures to SWCNT, MWCNT, or CNF. Generally, these reviews reported high-quality evidence of worker exposures. Coalescing the hazard potential assessed from animal toxicity data and the mounting evidence of workplace exposures has naturally led to the initiation and completion of several epidemiologic studies aiming to establish if an association exists between exposures to CNT/F and potential health effects or markers of early effect. However, many of the previous epidemiologic studies either have collected limited exposure data or were conducted within a single facility and enrolled few participants, ultimately limiting their overall generalizability ([Liou et al., 2015](#)).

The purpose of this paper is to present the results of an extensive exposure assessment monitoring study of workers with heterogeneous levels of exposure to CNT/F for an epidemiologic exposure-response analysis. Participants were recruited from across the US as part of a cross-sectional epidemiologic investigation of early effect markers. Occupational exposures were assessed using a multi-metric approach to characterize personal, full-shift exposures for each participant to a variety of CNT/F materials.

2. Methods

2.1. Facilities and participants

A total of 12 site visits were conducted between December 2012 and September 2014 to facilities across the US that produce or use CNT/F. In total, 108 workers were enrolled into the cross-sectional epidemiologic and exposure assessment study, which included workers exposed to CNT/F from various departments and job titles. Each facility had been previously assessed by researchers of the National Institute for Occupational Safety and Health (NIOSH) prior to this study ([Evans et al., 2010](#); [Methner et al., 2010](#); [Birch et al., 2011](#); [Dahm et al., 2015](#); [Dahm et al., 2012](#)).

General information was collected for each of the 12 facilities and tabulated in [Table 1](#). This information includes types of CNT/F material handled, material characteristics, observed daily quantities, company size, job tasks performed, as well as engineering controls and personal protective equipment (PPE) used. Additionally, each site was categorized into one of three distinct industries ([Dahm et al., 2015](#)), which include:

1. *Primary Manufacturers* – produced SWCNT or MWCNT and subsequently sold their products for research/industrial uses.
2. *Hybrid Producers/Users* – produced MWCNT or CNF and subsequently incorporated them into a product within the same facility.
3. *Secondary Manufacturers* – manufactured various composites which utilized SWCNT, MWCNT, or CNF within the composites/thermoplastics industries. A single secondary manufacturer in the electronics industry was included in this group as well.

2.2. Personal sampling and data collection

The sampling strategy focused on determining personal exposure to CNT/F for each participant over two full work-shifts. As this was a cross-sectional study, two days of personal sampling data were collected and averaged for each participant to limit daily exposure variations and provide more precise exposure estimates. Personal exposures were assessed based on the mass of airborne elemental carbon (EC) at both the respirable and inhalable aerosol particle size fractions, as well as characterizing airborne CNT/F exposure by transmission electron microscopy (TEM) ([Dahm et al., 2015](#)). All three collected personal samples were co-located, with sample inlets positioned together in order to sample the same air space.

Additionally, dermal hand and wrist exposures were assessed by

scanning electron microscopy (SEM) and sputum samples were analyzed by enhanced darkfield microscopy to confirm internal exposures to CNT/F. Direct-reading instruments were also used to collect data for the potential confounders of ultrafine and fine particle number as well as particle mass (PM 2.5) for the epidemiologic analysis.

Furthermore, information on current and past work histories obtained via in-person interviews with each participant included information such as: current and past job titles, tasks, and workplace co-exposures ([Schubauer-Berigan et al. \(in preparation\)](#)). Worker observation forms regarding daily performed tasks were completed through a combination of workplace observations and a short in-person interview by each participant at the end of each sampled work-shift (see Supplemental Material Appendix I & II). Job titles provided by each participant were grouped, by industry, into one of six general titles which included: Administrator, Chemist, Engineer, Maintenance, Research and Development (R & D), and Technician. The job title groupings were based upon reported and observed daily tasks performed as well as reported job titles. Also, a company-specific information form regarding company policy on PPE and engineering control uses was completed for each site after an interview with the facility or production manager.

2.3. Elemental carbon analyses of air samples

Personal respirable aerosol collection for EC was performed using 25-mm cassettes with quartz fiber filters (QFF) attached to GK 2.69 BGI cyclones (BGI Inc., Waltham, MA, USA) and Airchek XR5000 sampling pumps (SKC Inc., Eighty Four, PA, USA) operating at the cyclone specified flow rate of 4.2 liters per minute (lpm). Customized adapters for the GK 2.69 cyclone (BGI Inc.; catalog number 3503) were used to fit 25-mm cassettes. Personal samples were also collected using open-faced 25-mm cassettes and QFFs (SKC Inc.) to provide an estimate of the inhalable size fraction, as noted in [Dahm et al. \(2012\)](#), using Leland Legacy™ pumps (SKC Inc.) operating at 5.5–6 lpm.

Daily outdoor and/or indoor background measurements for the airborne mass of EC at the respirable and inhalable fractions were collected at each facility to assess the potential for interference by anthropogenic sources. These sources could include diesel exhaust, emissions from coal or oil-fired power plants, and the seasonal burning of biomass ([Schauer, 2003](#)). Locations for background sampling were selected based on professional judgment and knowledge of each facility.

The airborne mass concentration of EC was measured using the NIOSH Manual of Analytical Methods (NMAM) Method 5040 ([NIOSH, 2006a](#)), based on a thermal-optical analysis technique for organic and elemental carbon (OC and EC). As described previously ([Birch et al., 2011](#); [NIOSH, 2013](#); [Dahm et al., 2015](#); [Fatkhutdinova et al., 2016](#); [Shvedova et al., 2016](#)), for application to CNT/F, a manual assignment of the OC-EC 'split' was made. Bulk samples of the CNT/F materials, where available, were analyzed to obtain their thermal profiles. Manual splits were then assigned based on results for the bulk and background samples ([NIOSH, 2013](#); [Dahm et al., 2015](#); [Birch, 2016](#)). The reported limit of detection (LOD) for NMAM 5040 ranged from 0.1 to 0.9 µg EC per cm² of filter deposit, with an average of 0.39 µg/cm². Based on the total mass of respirable/inhalable EC collected on the filter, and the collected air volume, the respirable/inhalable EC concentration (µg/m³) was calculated.

2.4. Electron microscopy analyses of air samples

Personal samples were collected to approximate the inhalable size fraction using open-faced 25-mm cassettes with mixed cellulose ester filters (0.8 µm pore size; SKC Inc.) and Airchek XR5000 pumps (SKC Inc.) operating at 3.5–4 lpm. The samples were subsequently analyzed on a JEOL2100F transmission electron microscope (JEOL USA, Inc., Peabody, MA, USA) using a modified NMAM 7402, asbestos by TEM ([NIOSH, 2006b](#); [Birch et al., 2017](#)). Modifications relate to counting CNT/F particles, which were observed mainly as agglomerated

Table 1
Facility and material characteristics.

Facility ^a	Industry	Material Type ^b	Average CNT/F Diameter (nm)	Average CNT/F Length (µm)	Company Size	Observed Quantity Handled	Primary Form(s) of Material(s)	General Job Tasks Performed	Personal Protective Equipment ^c	Engineering Controls
1	Secondary	MWCNT(s)	57	400	< 10	10–100 g	Dispersed in Liquid and Embedded in Composite	Weighing/Sonicating, MWCNT Sheet Manufacturing, Composite Sanding, Office Work	N-100 FF, Lab Coat	Clean Room, LEV, CFH
2	Hybrid	CNF(s), MWCNT	110	200	< 10	> 1 kg	Dry Powder	Sieving, Packaging/Shipping, Quality Control Testing, CNF/Resin Extrusion, Office Work	Full/Half Face Resp w/P-100, Tyvek Suits, Lab Coats	Enclosed Production, LEV, CFH
3	Secondary	CNF, MWCNT(s)	N/A	N/A	< 10	> 1 kg	Dry Powder	MWCNT Weighing, Functionalization of CNF, Office Work	Full Face Resp. w/P-100, Tyvek Suits	Clean Room, LEV, Enclosed Functionalization
4	Primary	MWCNT(s)	17.5	30	< 10	10–100 g	Dry Powder	Weighing/Sieving/Sonication, Production & Harvesting, Office Work	Half Face Resp w/P-100, HEPA Glovebox, CFH, VE	HEPA CFH, Ventilated Glove Box
5	Secondary	MWCNT(s), SWCNT	N/A	N/A	10–30	< 10 g	Dispersed in Liquid	Weighing, SWCNT Ink Production, MWCNT Purification, Sonication, Office Work	Lab Coats	HEPA CFH, Ventilated Glove Box
6	Secondary	MWCNT(s)	N/A	N/A	> 100	> 1 kg	Dry Powder and Embedded in Composite	Weighing, Extrusion of MWCNT/Resin Mixture	Half Face Resp w/P-100, Tyvek Suits	CFH, LEV, VE
7	Secondary	MWCNT(s)	12.5	3.3	10–30	101 g–1 kg	Dry Powder and Embedded in Composite	Weighing/Mixing Dispersions, Composite Strength Testing, Composite Sanding, Quality Control Testing, Office Work	Full Face Resp w/N-100, Tyvek Suits	HEPA CFH, LEV
8	Primary	MWCNT(s)	59	1025	10–30	101 g–1 kg	Dry Powder	Production & Harvesting, R & D Production, Wet Lab Work, Packaging/Shipping, Office Work	Half Face Resp w/P-100, Lab Aprons, Face Shields	CFH, LEV, Enclosed Production
9	Primary	SWCNT(s), MWCNT(s)	N/A	N/A	10–30	> 1 kg	Dry Powder and Dispersed in Liquid	MWCNT/SWCNT Production/Purification, Packaging/Shipping, Quality Control Testing, Office Work	Half/Full Face Resp w/P-100, Lab Coats, Tyvek Suits	Glove Boxes, LEV, Dust Collection System, CFH
10	Primary	SWCNT, MWCNT	1	1.5	10–30	10–100 g	Dry Powder and Dispersed in Liquid	Production & Harvesting, Wet Lab Work, Office Work	N-95 and P-100 FF, Lab Coats	Enclosed Production, CFH
11	Hybrid	MWCNT(s)	1.5	65	31–100	> 1 kg	Dry Powder and Embedded in Composite	Production, Post-Coating, Quality Control Testing, Office Work	N-95 FF, Full, and Half Face Resp w/P-100, Tyvek Lab Coats	Enclosed Production, LEV, HEPA CFH, VE
12	Hybrid	MWCNT(s), SWCNT	7.5	1000	31–100	> 1 kg	Dry Powder and Embedded in Composite	Production & Harvesting, Maintenance of Reactors, Post Processing, Quality Control Testing, Office Work	Full and Half Face Resp w/P-100, Tyvek Lab Coats	Enclosed Production, VE, HEPA LEV

MWCNT = multi-walled carbon nanotube; SWCNT = single-walled carbon nanotube; CNF = carbon nanofiber; N/A = not available; Resp = respirator; FF = filtering facepiece respirator; LEV = local exhaust ventilation; CFH = chemical fume hood; VE = ventilated enclosure; HEPA = high-efficiency particulate air.

^a Facility ID corresponds to same IDs in Schubauer-Berigan et al. (in preparation).

^b Material Type listed first indicates the material predominantly observed being used during the site visit.

^c All Facilities used Nitrile Gloves and Safety Glasses as Personal Protective Equipment.

structures rather than individual fibers. Given the structural differences, the following requirements for asbestos fiber counts were eliminated: 1.) the requirement that counted particles have diameters $> 0.25 \mu\text{m}$ and 2.) that particles meet the traditional definition of a fiber (aspect ratio $\geq 3:1$ and fiber length $> 5 \mu\text{m}$). Particles containing CNT/F were counted as CNT/F ‘structures’, which ranged from single-CNT/F fibers to various sized agglomerates comprised of many CNT/F fibers. All structures, agglomerated or single fibers, were counted as one CNT/F structure. Based on the total number of CNT/F structures counted, and the collected air volume, the total CNT/F structures per cm^3 concentration (s/cm^3) was calculated (Dahm et al., 2015; Birch et al., 2017).

CNT/F structures were also categorized by size-bins for each sample based on a visual estimate of structure size and degree of agglomeration (Dahm et al., 2015; Birch et al., 2017). Most agglomerated CNT/F particles were roughly spherical (i.e., the longest dimension was no more than two times the length of the orthogonal dimension); therefore, the maximum crosswise dimension was used to categorize the structure’s size. The counted CNT/F structures were placed into six discrete size-bins which included a single CNT/F fiber bin, CNT/F structure agglomerates with ‘diameters’ (maximum crosswise dimension) $< 1 \mu\text{m}$, agglomerates with diameters between 1 to $< 2 \mu\text{m}$, agglomerates between 2 to $< 5 \mu\text{m}$, agglomerates between 5 to $< 10 \mu\text{m}$, and agglomerates with diameters $> 10 \mu\text{m}$. Size bin specific concentrations, as CNT/F s/cm^3 , were calculated for the following sizes: single fibers, CNT/F agglomerates $< 1 \mu\text{m}$, $< 2 \mu\text{m}$, $< 5 \mu\text{m}$, and $< 10 \mu\text{m}$ based upon the count of CNT/F structures at that bin as well as the corresponding bins below.

2.5. Dermal analyses

Dermal samples were collected post-shift on one day of sampling for each individual participant and subsequently analyzed by scanning electron microscopy (SEM). Two samples per participant were collected using a tape stripping method upon the outermost skin layer, the stratum corneum (Schneider et al., 2000; Lundgren et al., 2006). Transparent adhesive tape (3 M St. Paul, MN, USA) with a width of 19 mm and length of ~ 4 cm was used to collect each sample. One sample each was collected from the worker’s dominant hand and wrist. Hand samples were collected from the worker’s fingertips and palm while wrist samples were collected on the underside of the wrist.

The sticky surface of the tape was pressed against the worker’s skin in the area to be sampled, and rubbed lightly to assure adhesion before being gently removed. This was repeated on the hand and wrist, respectively, for ~ 30 s each. After each sample collection, the tape strip was folded into itself and placed in a small petri dish for storage and transport. SEM analysis was then used to visually identify CNT/F structures in a qualitative fashion, indicating either the presence or absence of CNT/F.

For analysis, each tape sample was placed into a crucible and ashed at 480°C for 4 h. After cooling, the crucibles were filled with isopropyl alcohol and sonicated for 10 min. The liquid was then filtered onto a $0.4 \mu\text{m}$ polycarbonate filter and a portion of each filter was placed onto a carbon-taped stub and gold coated. The samples were analyzed at 10,000X by a Tescan SEM (TESCAN, Brno, CZ) equipped with a Gresham light element detector.

2.6. Sputum analyses

Induced sputum was collected for each consenting participant mid-week during the participant’s work-shift. A detailed summary of sputum collection, handling, post-processing, and dark-field microscopy analysis can be found in Beard et al. (in preparation), (Schubauer-Berigan et al. (in preparation), and Mercer et al. (2011,2017)). In brief, each study participant inhaled a sterile isotonic saline solution produced by a compressed-air nebulizer for a total of 12 min. Every two minutes, the

participant would take a deep breath and cough the full breath along with the sputum into a sputum cup for collection. The specimens were post-processed and centrifuged into an acellular and cellular fraction within an hour of collection and shipped on dry ice to the lab for analysis.

The cellular fraction was examined using enhanced dark-field microscopy for evidence of CNT/F following procedures established by Mercer et al. (2011,2017). Approximately 3000 cells per slide (0.5–1% of the collected sputum sample) were viewed. Prior to analysis, a cytospin of the cell pellet was prepared on ultrasonically cleaned, laser cut slides (Schott North America, Inc, Elmsford, N.Y. 10523), to avoid nanoparticle contamination from the ground edges of traditional slides and stained with Sirius Red-Hematoxylin. The optical system consisted of high signal-to-noise, darkfield-based illumination optics adapted to an Olympus BX-41 microscope (CytoViva, Auburn, AL 36830). After alignment of the substage oil immersion optics with a 10X objective, sections were examined with 60X air or 100X oil immersion objectives. CNT/F structures were then qualitatively characterized as present or absent within each sample.

2.7. Direct-reading instruments

Most ultrafine/fine particles found within CNT/F workplaces have been attributed to incidental aerosols from anthropogenic sources (Maynard et al., 2004; Evans et al., 2010; Dahm et al., 2013). Examples include vehicle exhaust, reactor byproducts, industrial dryers, compressors, and vacuum cleaners, which can contribute to the total ultrafine/fine particle concentration. Therefore, it was deemed prudent to collect representative area samples to measure ultrafine/fine particle number and particle mass (PM 2.5) which could act as confounding variables within the cross-sectional epidemiologic analysis.

Measurements of particle number concentration in the size range of 10–1000 nm were performed with a CPC (CPC 3007; TSI Inc., Shoreview, MN, USA) and recorded as the total number of particles per cubic centimeter (P/cm^3) of air. Particle number concentration and size distributions from 22.9 nm to $10 \mu\text{m}$ aerodynamic diameter were obtained with an ELPI (Dekati Ltd, Kangasala, Finland) and data are reported as P/cm^3 . Direct-reading mass estimates of PM 2.5 were obtained using a combined photometer-optical particle counter (OPC) (DustTrak DRX 8533, TSI Inc.) and data are reported in $\mu\text{g}/\text{m}^3$. A one second data logging interval was selected for the CPC, ELPI, and photometer-OPC. All three instruments were placed on a custom mobile particle measurement platform, described in Evans et al. (2010). The sampling platform was moved throughout each facility to sample representative areas where participants spent a majority of their workday.

2.8. Statistical analyses

EC mass and TEM structure concentrations were averaged for the two days of sampling for each participant. These averaged concentrations are reported throughout the manuscript. Day 2 samples could not be obtained for eight individuals, while three participants had a total of three to four days of samples collected due to the variety of tasks they performed within their workplace.

For direct comparison to the NIOSH respirable recommended exposure limit (REL) for CNT/F an 8-h time weighted average (TWA) is required (NIOSH, 2013). The average sampling time for all respirable EC was 476 min (for inhalable EC and TEM it was 474 min and 471 min, respectively). Therefore, it is assumed that the reported respirable concentrations were representative of 8-h samples, allowing for direct comparison to the REL. Exposure concentrations are summarized as arithmetic means (AM), geometric means (GM), geometric standard deviations (GSD), and the 10th and 90th percentile of the exposure distributions.

For all personal samples, EC concentration values below the LOD

Table 2
Current and past self-reported nanomaterial exposures and co-exposures.

Material Category	Material	Percent Exposed	Percent with Past Exposure
Nanomaterials	MWCNT	68	15
	SWCNT	24	11
	CNF	7	4
	Other Nano ^a	15	13
Particulates & Dusts	Polymers	27	27
	Asbestos	0	4
	Other Dusts ^b	22	50
Fumes, Vapors, & Mists	Paint	1	8
	Metal Working Fluid	0	7
	Other Fumes ^c	1	7
	Solvents ^d	52	47
Acids, Bases, & Solvents	Solvents ^d	52	47
	Strong Acids	21	22
	Strong Bases	6	6

MWCNT = multi-walled carbon nanotube; SWCNT = single-walled carbon nanotube; CNF = carbon nanofiber.

^a Other Nano includes: boron nitride, graphene, quantum dots, fullerenes, nano-silver, nano-silicon, nano-iron, nano-gold, unspecified nano-metal oxides, nano-clay, nano-nickel.

^b Other Dusts includes: silica, powdered carbon, diesel exhaust (grouped with other dusts due to similar lung health effects), and non-specified dusts.

^c Other Fumes Includes: welding and other non-specified metal fumes.

^d Solvents Includes: alcohol, methyl ethyl ketone, dimethylformamide, dimethylacetamide, acetone, xylene, trichloroethylene, hexamethyldisilazane, toluene, methylene chloride, hexane, benzene, *n*-methyl-2-pyrrolidone, naphthalene, *n*-pentane, tetrahydrofuran.

[$n = 42$ (19.6%), respirable; $n = 17$ (7.9%), inhalable] were substituted with values equal to one-half the LOD determined at a specific facility (Hornung and Reed, 1990). The EC data were then background-corrected to adjust for anthropogenic sources of EC by subtracting the facility-specific background EC concentration, by type of sample (respirable or inhalable) and sampling day, before being averaged. In instances where a negative background-corrected concentration was found (background concentration > sample concentration; [$n = 99$ (46.3%), respirable; $n = 91$ (42.5%), inhalable]), 1/2 the lowest background adjusted value from the specific facility was substituted (Dahm et al., 2015). Each imputed data point was reviewed on a facility-by-facility basis for validation.

The TEM concentrations that fell below the LOD [$n = 50$ (23.4%)], which was defined as 1 CNT/F structure per filter, were substituted with one-half the LOD (0.5 structures per filter). Subsequently, the concentration was calculated using the air volume for the specific non-detectable sample. Samples overloaded with material [$n = 16$ (7.5%)] were verified to contain heavy loadings of CNT/F by TEM, and carbon by energy dispersive X-ray spectroscopy, but structure counts could not be performed for these samples. Therefore, counts were estimated by assuming overloaded samples were 50% higher than the largest CNT/F structure count found at that specific facility. The concentration was then calculated using this estimated structure count along with the air volume of the overloaded sample.

A daily weighted value for sampling Days 1 and 2 were derived for ultrafine/fine particle concentration and PM 2.5 mass using the three direct-reading instrument outputs. The weighted values were based on the amount of time workers spent during their work shifts in parts of the facility where a general area sample was collected, and then averaged over the two days for a single representative value. The ELPI impactor cut-points of 22.9–95.8 nm were selected to represent the ultrafine particle concentration, which also had a very high correlation with the remaining impactor stages up to 10 μm (Pearson $r > 0.99$ for each stage) and therefore is the only size fraction presented.

The statistical analyses were performed on the log-transformed data. Correlations between EC mass, TEM size fractionated and total concentrations, and direct-reading instrument results in paired samples

were examined using the non-parametric Spearman rank correlation coefficient. The non-parametric Kruskal-Wallis test was used to determine significant differences between job titles and self-reported exposures while the Wilcoxon signed-rank test was used to compare each participant sample results collected on Day 1 to their Day 2 sampling results for EC and TEM total concentrations. Non-parametric tests were used to accommodate the few exposure distributions that were non-normal after the log transformation. Logistic regression models were also created to determine if increased exposure to CNT/F, as measured by respirable EC, Inhalable EC, and total TEM concentrations, could predict an increase in the probability of detecting dermal and sputum exposures. Statistical significance was evaluated based on $p = 0.05$ (two-sided) for all tests. All statistical analyses were performed using SAS version 9.3 (Cary, NC, USA).

3. Results

3.1. Facility and worker level observations

An overview of facility and CNT/F characteristics, including general job tasks and exposure control measures, are provided in Table 1. Information on cohort demographics are located in Schubauer-Berigan et al., while information on exposures by demographic group can be found in the Supplemental Material, see Table S1. The participating facilities were generally small to medium sized employers with nine of the 12 facilities employing fewer than 30 employees. Four of the 12 facilities were primary manufacturers, three were hybrid producers and users, and the remaining five facilities were considered secondary manufacturers. Most facilities predominantly used MWCNT materials during the days of observation, with a wide range of average diameters (1.5–110 nm) and lengths (3.3 μm –1 mm). Daily quantities produced or used were > 1 kg for five of the eight secondary and hybrid manufacturers, whereas two of the four primary manufacturing typically produced < 100 g of material per day. All but two of the facilities handled CNT/F in the dry powdered form for some portion of the day.

Information on current and past self-reported nanomaterial exposures, as well as co-exposures, were collected from each participant and organized in Table 2. Nanomaterial exposures of the 108 participants were predominantly to MWCNT followed by SWCNT, other nanomaterials, and CNF. The average cumulative duration of exposure for the 108 participants to MWCNT was 7.8 years, while cumulative durations for CNF, SWCNT, and other nanomaterials were 7.5, 5.1, and 3.9 years, respectively. Co-exposures included a variety of particulate, dust, fume, vapor, mist, acid/base, and solvent exposures. Most current co-exposures were to various solvents (52%), polymers (27%), other dusts (22%), and strong acids (21%). Similarly, most past exposures were to other dusts (50%), solvents (47%), polymers (27%), and strong acids (22%). A significant difference was found between the current self-reported exposure status of exposed compared to the non-exposed status for MWCNT at the inhalable size fraction. However, no other significant differences were found between the self-reported exposure statuses of SWCNT, CNF, and the remaining co-exposures (Supplemental Material, see Table S2).

3.2. Overall and averaged exposures for EC/TEM

In total, 214 samples were collected between multiple days of sampling for the mass of EC at the respirable and inhalable aerosol fractions, as well as for TEM (inhalable fraction) analysis, from all 108 participants (Table 3). The overall arithmetic mean (AM) exposure for the respirable fraction of EC was 1.35 $\mu\text{g}/\text{m}^3$, with values ranging from 0.001 $\mu\text{g}/\text{m}^3$ to 80.30 $\mu\text{g}/\text{m}^3$, while the inhalable EC exposure AM was 7.68 $\mu\text{g}/\text{m}^3$, with values ranging from < 0.001 $\mu\text{g}/\text{m}^3$ to 709.30 $\mu\text{g}/\text{m}^3$. Total AM TEM exposures were 0.1913 CNT/F s/cm³, with values ranging from < 0.0001 to 5.4654 s/cm³.

Averaging EC mass concentrations for Day 1 and 2 of sampling for

Table 3
Overall and averaged EC mass, total TEM, dermal, and sputum exposures.

		EC						TEM					Dermal		Sputum	
		n	AM ($\mu\text{g}/\text{m}^3$)	GM ($\mu\text{g}/\text{m}^3$)	GSD	10th PCTL	90th PCTL	n	AM (s/ cm^3)	GM (s/ cm^3)	GSD	10th PCTL	90th PCTL	% Pos. Wrist	% Pos. Hand	% Pos.
Total Samples	Resp.	214	1.35	0.06	10.87	< 0.01	1.27	–	–	–	–	–	–	–	–	–
Collected	Inhal.	214	7.68	0.11	31.57	< 0.01	3.65	214	0.1913	0.0054	17.75	0.0001	0.1846	–	–	–
Averaged	Resp.	108	1.00	0.08	8.99	< 0.01	0.80	–	–	–	–	–	70	63	18	
Exposures	Inhal.	108	6.22	0.14	23.77	< 0.01	3.56	108	0.1275	0.0079	13.92	0.0002	0.1955	–	–	–

EC = elemental carbon; TEM = transmission electron microscopy; AM = arithmetic mean; GM = geometric mean; GSD = geometric standard deviation; PCTL = percentile; Pos. = positive.

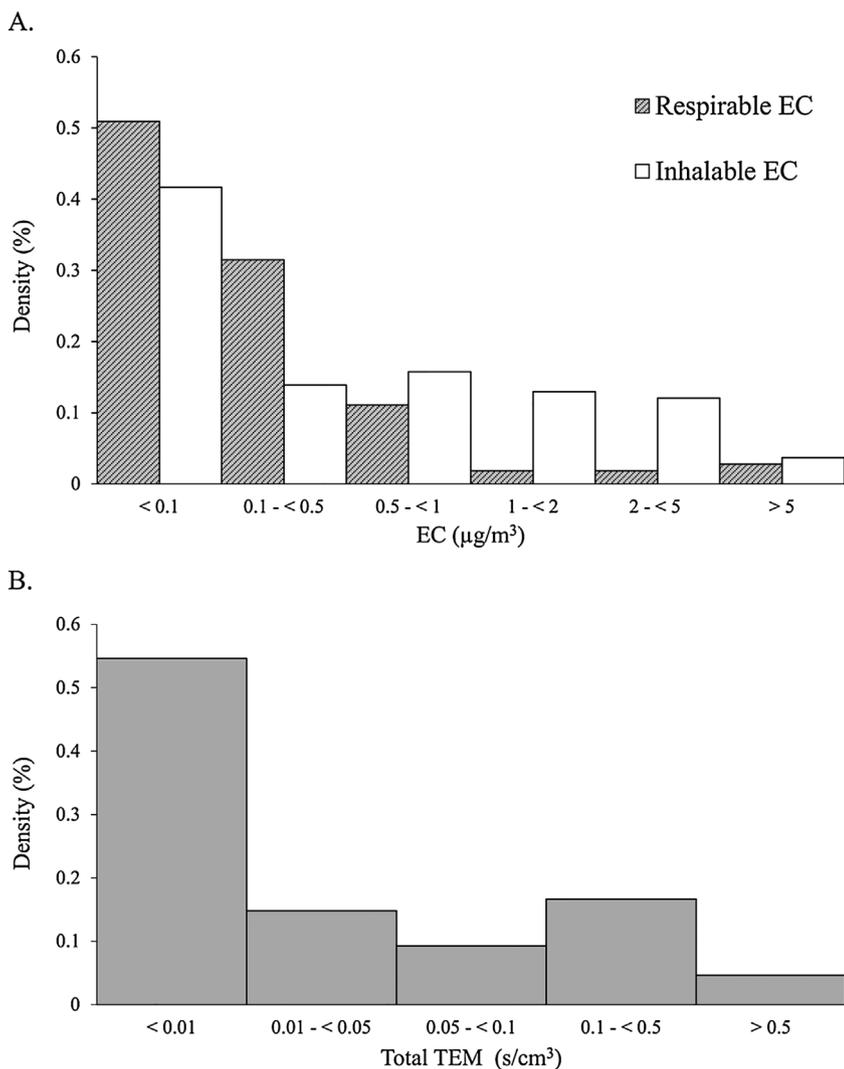


Fig. 1. Exposure distributions for A.) averaged respirable and inhalable EC mass concentrations and B.) averaged total TEM concentrations for all 108 participants. EC = elemental carbon; TEM = transmission electron microscopy.

the 108 participants yielded an AM exposure of $1.00 \mu\text{g}/\text{m}^3$ (range; $0.001\text{--}43.80 \mu\text{g}/\text{m}^3$) and $6.22 \mu\text{g}/\text{m}^3$ (range; $< 0.001\text{--}417.91 \mu\text{g}/\text{m}^3$) for the respirable and inhalable fractions, respectively. Averaged total TEM exposures ranged from 0.0001 to $3.5416 \text{s}/\text{cm}^3$ with an AM of $0.1275 \text{s}/\text{cm}^3$. Respirable and inhalable EC mass concentration data as well as the TEM structure concentrations showed a right skewed distribution (Fig. 1A and B). Approximately 7% of the averaged EC mass values collected at the respirable fraction and 29% at the inhalable fraction were found to be above $1 \mu\text{g}/\text{m}^3$ (Fig. 1A). Meanwhile, 45% of the averaged total TEM concentrations were $> 0.01 \text{s}/\text{cm}^3$ and 21% were $> 0.1 \text{s}/\text{cm}^3$ (Fig. 1B). Additionally, no significant differences were found between the samples collected on Days 1 and 2 for

respirable EC mass, inhalable EC mass, and total TEM concentrations with *p*-values of 0.08, 0.86, and 0.17, respectively.

EC background sample concentrations collected at all 12 facilities over multiple days ranged from 0.02 to $4.06 \mu\text{g}/\text{m}^3$ with an AM of $0.64 \mu\text{g}/\text{m}^3$ at the respirable fraction, while the inhalable fraction ranged from 0.02 to $3.89 \mu\text{g}/\text{m}^3$ with an AM of $0.75 \mu\text{g}/\text{m}^3$. The maximum values obtained for both size fractions were from a single facility where diesel exhaust intrusion occurred via the building HVAC system. After removing these elevated values, the average respirable and inhalable background concentrations were lowered to 0.50 and $0.62 \mu\text{g}/\text{m}^3$, respectively.

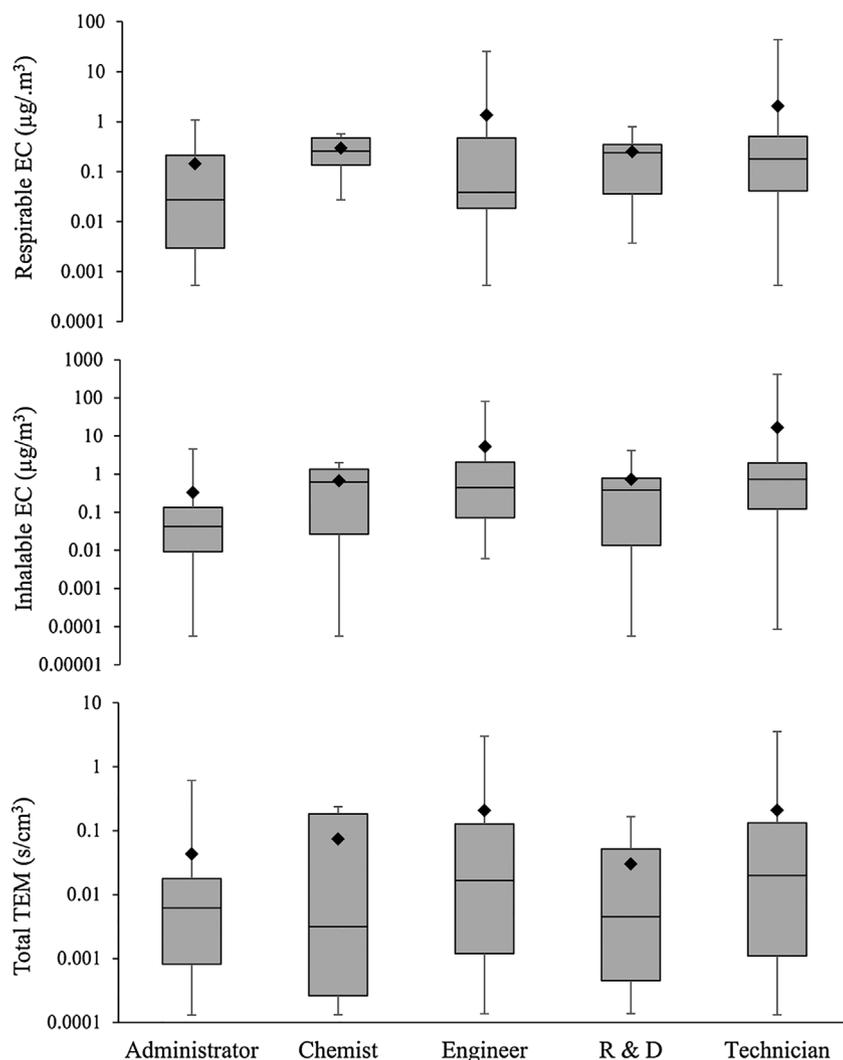


Fig. 2. Five number summary (minimum, first quartile, median, third quartile, and maximum) of average exposures for all 108 participants subdivided by overall job title. Maintenance job title was excluded due to limited sample size. ♦ denotes arithmetic mean value. A significant difference ($p < 0.05$) was found between the Administrator job title (control) and the chemist, R & D, and technician job titles at the respirable EC fraction; engineer and technician at the inhalable EC fraction; while no significant difference were observed for TEM. Significant differences between job titles were based on non-parametric Kruskal-Wallis test.

3.3. EC/TEM exposures by job title and industry

Job titles were grouped into 6 standardized designations, seen in Fig. 2. Overall, significant differences were found between job titles and the exposure metrics of respirable ($p = 0.03$) and inhalable EC ($p < 0.01$), while no association was found between job titles and total TEM concentration ($p = 0.71$). When examining individual job titles, using Administrators as a control group, significant differences were found between Administrators and Chemists ($p = 0.04$), R&D workers ($p = 0.02$), and Technicians ($p = 0.01$) for respirable EC. Significant differences were also observed for the inhalable fraction of EC between Administrators and Engineers ($p = 0.001$) as well as Administrators and Technicians ($p = < 0.001$), but no significant differences were identified between Administrators and the other job titles when comparing total TEM values.

Job titles were also analyzed by industry (Table 4). At primary manufacturing facilities, no significant difference was found between job titles and respirable EC, inhalable EC, or the total TEM concentration ($p = 0.08$; 0.23; 0.33). The lowest averaged AM respirable and inhalable EC mass exposures were associated with the standardized job title of Engineer ($0.06 \mu\text{g}/\text{m}^3$) and Administrator ($0.67 \mu\text{g}/\text{m}^3$), respectively, while the highest exposures were seen among the R&D ($0.30 \mu\text{g}/\text{m}^3$, respirable) and Technician ($1.98 \mu\text{g}/\text{m}^3$, inhalable) job titles. The lowest total AM TEM exposures among the primary manufacturing job titles was found for R&D workers ($0.0337 \text{ s}/\text{cm}^3$) while the highest exposure was seen among Technicians ($0.0867 \text{ s}/\text{cm}^3$).

Hybrid facilities did not show significant differences between job titles and any of the three exposure metrics (respirable EC, $p = 0.26$; inhalable EC, $p = 0.11$; total TEM, $p = 0.64$). At secondary manufacturing facilities, a significant difference was found between job titles and respirable ($p = 0.03$) and inhalable ($p = 0.04$) EC while no difference was observed for total TEM concentration ($p = 0.68$). Administrators had the lowest AM exposures to respirable EC (0.04 ; $0.05 \mu\text{g}/\text{m}^3$) and inhalable EC (0.06 ; $0.08 \mu\text{g}/\text{m}^3$) and were among the lowest total TEM concentrations at both the hybrid and secondary manufacturing facilities. Technicians had the highest respirable ($3.66 \mu\text{g}/\text{m}^3$) and inhalable ($30.57 \mu\text{g}/\text{m}^3$) AM EC exposures among the hybrid facilities, while Engineers had the highest respirable ($0.56 \mu\text{g}/\text{m}^3$) and R&D workers had the highest inhalable ($0.62 \mu\text{g}/\text{m}^3$) AM EC among secondary manufacturers. The highest total TEM concentrations were seen among Technicians ($0.3433 \text{ s}/\text{cm}^3$) and Engineers ($0.0495 \text{ s}/\text{cm}^3$) at the hybrid and secondary manufacturing facilities, respectively.

3.4. Dermal and sputum exposures

Of the 108 employees who participated in the study, six participants did not provide dermal samples of the wrist or hand and 18 participants did not provide a sputum sample. Overall, from the 102 participants that provided dermal samples, 70% showed the presence of CNT/F on the wrist while 63% of the samples showed the presence of CNT/F on the hand. Meanwhile, from the 90 participants that provided a sputum sample, only 18% had CNT/F present in the sputum. When subdivided

Table 4
Averaged industry EC mass, total TEM, dermal, and sputum exposures by collapsed job title.

	Job Title	n	Respirable EC			Inhalable EC			TEM			Dermal		Sputum
			AM (µg/m ³)	GM (µg/m ³)	GSD	AM (µg/m ³)	GM (µg/m ³)	GSD	AM (s/cm ³)	GM (s/cm ³)	GSD	% Pos. Wrist	% Pos. Hand	% Pos.
Primary Manufacturer (n = 4)	Administrator	12	0.27	0.08	11.34	0.67	0.01	86.14	0.0723	0.0044	18.42	45	73	0
	Chemist	5	0.29	0.21	3.25	0.68	0.08	69.95	0.0742	0.0055	28.98	25	25	20
	Engineer	7	0.06	0.02	9.59	0.68	0.11	14.00	0.0553	0.0337	3.74	43	43	33
	R & D	8	0.30	0.23	2.53	0.93	0.03	101.97	0.0337	0.0031	12.68	88	50	14
Hybrid Producer/User (n = 3)	Technician	7	0.24	0.09	10.85	1.98	0.45	46.62	0.0867	0.0319	12.65	57	29	33
	Administrator	4	0.04	0.03	2.32	0.06	0.06	1.00	0.0014	0.0011	2.63	75	25	0
	Engineer	15	2.16	0.14	10.55	8.80	0.69	12.30	0.3184	0.0124	21.82	87	67	14
	Maintenance	2	0.52	0.43	2.38	0.93	0.68	3.18	0.0448	0.0065	40.20	100	100	0
Secondary Manufacturer (n = 5)	R & D	2	0.15	0.10	3.72	0.32	0.10	13.41	0.0215	0.0024	58.11	100	100	0
	Technician	16	3.66	0.24	8.71	30.57	1.06	11.08	0.3433	0.0124	23.43	94	75	14
	Administrator	12	0.05	< 0.01	5.89	0.08	0.03	4.32	0.0278	0.0094	4.37	56	67	11
	Engineer	4	0.56	0.08	12.71	0.38	0.26	3.02	0.0495	0.0016	29.86	75	100	0
	R & D	7	0.22	0.06	8.00	0.62	0.26	5.20	0.0282	0.0085	6.52	50	67	67
	Technician	7	0.25	0.09	6.22	0.57	0.10	9.76	0.0192	0.0046	11.92	71	71	20

EC = elemental carbon; TEM = transmission electron microscopy; AM = arithmetic mean; GM = geometric mean; GSD = geometric standard deviation; PCTL = percentile; Pos. = positive; R & D = research and development.

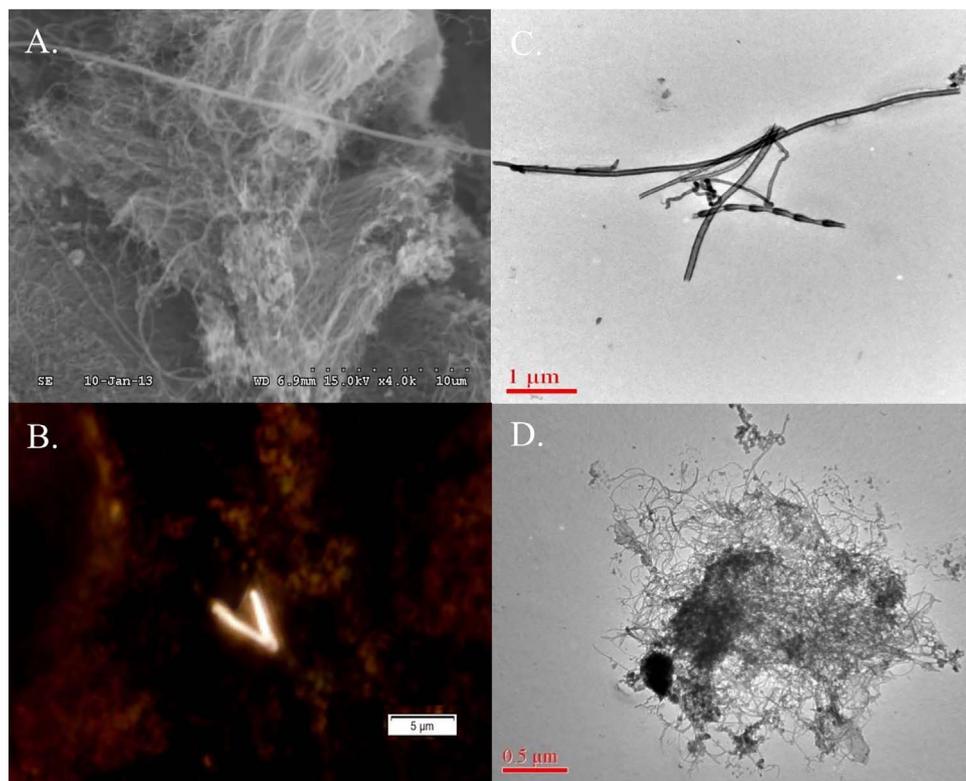


Fig. 3. Electron microscopy and enhanced darkfield images from (A) SEM dermal wrist sample of MWCNT agglomerate. (B) Enhanced darkfield microscope image of MWCNT in sputum. (C) TEM personal air sample showing CNF Single fibers. (D) TEM personal air sample of MWCNT 2–5µm agglomerate.

by industry, positive dermal exposures of the wrist and hand were found for 54% and 49% for participants working within primary manufacturing facilities, 90% and 69% among hybrid facilities, and 62% and 73% among participants within secondary manufacturing facilities. Likewise, 18%, 11%, and 27% of the participants within the primary manufacturing, hybrid, and secondary manufacturing facilities had CNT/F present in their sputum. Examples of particles found from the dermal and sputum analyses can be seen in Fig. 3A and B.

Logistic regression models were created to predict the presence/absence of CNT/F from dermal samples collected on the wrist and hand as well as the presence/absence of CNT/F in sputum based upon increasing exposures from respirable or inhalable EC and total TEM concentrations. Two of the six models created to predict dermal exposures were found to be significant which included respirable EC

predicting the presence/absence of dermal wrist CNT/F exposure ($p = 0.001$) and total TEM concentration predicting the presence/absence of dermal hand CNT/F exposure ($p = 0.049$). However, we found an inverse relationship between the total TEM concentration and dermal hand exposure. Two other dermal exposure models revealed borderline significant results, which included inhalable EC predicting dermal wrist exposure ($p = 0.053$) and respirable EC predicting dermal hand exposures ($p = 0.053$). None of the three predictive models identified a relationship between increasing exposures for respirable EC, inhalable EC, and total TEM concentration ($p = 0.82, 0.30, \text{ and } 0.15$; respectively) and the presence/absence of CNT/F in sputum.

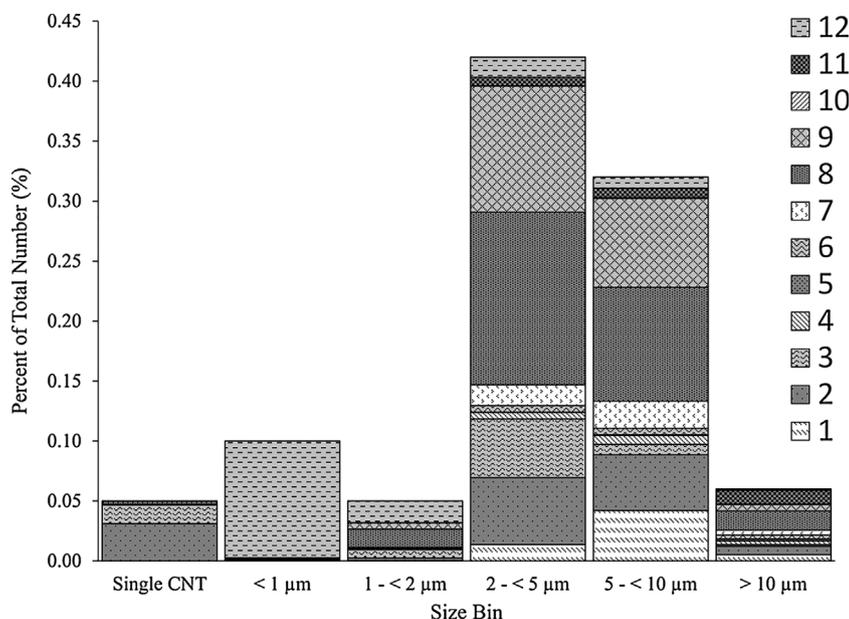


Fig. 4. Percentages of the averaged CNT/CNF structures counted per size bin for each of the 108 participants. Each size bin percentage is subdivided by the contribution made from each facility (1–12). “Single CNT” size bin includes all non-agglomerated single CNT fibers.

3.5. TEM size distribution analysis

CNT/F structures were categorized by TEM analysis into discrete size-bins based on a visual estimate of structure size and degree of agglomeration. Fig. 4 displays the particle size distribution based on the percentage of the total averaged CNT/F structures counted per size bin for the 108 participants subdivided by facility. Single CNT/F fibers (Fig. 3C) and agglomerates < 1 μm, 1 to < 2 μm, and > 10 μm were uncommon, while approximately 75% of the CNT/F structures counted represented agglomerates of 2–10 μm (Fig. 3D).

Correlations between the averaged size-bin specific TEM concentrations and averaged EC concentrations at both the respirable and inhalable fractions for the 108 participants were compiled in Table 5. The highest correlations observed for respirable EC were found at the lower-sized bins, more likely to be included in the respirable fraction (single-CNT/F fiber bin, $p = 0.32$; < 1 μm, $p = 0.39$; and < 2 μm, $p = 0.30$), while decreasing correlations were seen at the larger-size bins, more likely to be included in the thoracic and inhalable size fractions (< 5 μm, $p = 0.25$; < 10 μm, $p = 0.22$), and between the total TEM concentration ($p = 0.21$). However, all correlations were found to be significant. Correlations between inhalable EC and the various TEM size-bin specific concentrations ($p = 0.39$ – 0.45) and the total TEM concentration ($p = 0.47$) showed a tendency for higher correlation coefficients among the larger size bins (Table 5).

3.6. Direct-reading instrument confounding exposures

A wide variation was seen in the weighted averages by facility,

Table 5 Spearman correlation coefficient (p -value) between averaged TEM size bin and averaged inhalable and respirable EC mass.

TEM Bin	Elemental Carbon	
	Respirable	Inhalable
Single Fibers	0.32 (0.0008)	0.30 (0.0016)
< 1 μm	0.39 (< 0.0001)	0.42 (< 0.0001)
< 2 μm	0.30 (0.0017)	0.39 (< 0.0001)
< 5 μm	0.25 (0.0095)	0.45 (< 0.0001)
< 10 μm	0.22 (0.0209)	0.45 (< 0.0001)
Total Conc.	0.21 (0.0309)	0.47 (< 0.0001)

TEM = transmission electron microscopy; Conc. = concentration.

especially for ultrafine and fine particle concentrations measured by the CPC and ELPI (Supplemental Material, see Fig. S2). Significant correlations were found between respirable EC and all three direct-reading instruments while inhalable EC significantly correlated with the CPC ($p = 0.52$) and ELPI ($p = 0.38$), but no significant association was found between inhalable and PM 2.5 (0.14), as seen in Table 6. Additionally, no significant correlations were found for the exposure metric of total TEM between the three direct-reading instruments.

4. Discussion

This study assessed exposure to CNT/F for 108 participants within 12 facilities located across the US as part of a cross-sectional epidemiologic investigation of early effect markers. Most facilities included in this study reported, and were observed, using MWCNT, compared to SWCNT and CNF materials. Nearly all facilities initially handled the dry powder form of the material, but then dispersed the CNT/F powders into a liquid solution or embedded them into a composite reducing the likelihood of exposure. While all facilities used some combination of PPE and exposure control measures to reduce the potential of exposure, the extent of their use and capabilities varied greatly from facility-to-facility.

Additionally, CNT/F diameters and lengths varied greatly between facilities, in some cases surpassing an order of magnitude difference. This is important to note because recent animal toxicity data has indicated increased toxicity with increasing CNT/F diameter and length (Sohaebuddin et al., 2010; Murphy et al., 2011; Hamilton et al., 2013; Manke et al., 2014).

Table 6 Spearman correlation coefficient (p -value) between averaged exposure metrics and direct-reading instruments.

Direct Reading Instrument	Exposure Metric		
	Respirable EC (μg/m ³)	Inhalable EC (μg/m ³)	Total TEM (s/cm ³)
CPC (p/cm ³)	0.46 (< 0.0001)	0.52 (< 0.0001)	0.18 (0.07)
ELPI 22.9–95.8 nm (p/cm ³)	0.47 (< 0.0001)	0.38 (< 0.0001)	0.02 (0.85)
DustTrak PM 2.5 (μg/m ³)	0.23 (0.02)	0.14 (0.14)	–0.13 (0.18)

EC = Elemental Carbon; TEM = Transmission Electron Microscopy.

Although the exposure data in this study follow a lognormal distribution, the AM, as opposed to the geometric mean (GM), was utilized to describe exposures for each worker. The AM is sensitive to outliers, but it remains the most frequently used metric because studies suggest that it better reflects cumulative exposure over a time period and/or population (Seixas et al., 1988). For this reason, the AM is often preferred over the GM in epidemiologic studies (Crump, 1998).

Approximately 7% of averaged AM samples collected at the respirable fraction for EC mass were found to be greater than the NIOSH REL of $1 \mu\text{g}/\text{m}^3$. Nearly 30% of the samples collected at the inhalable size fraction were $> 1 \mu\text{g}/\text{m}^3$, although there are no exposure limits for CNT/F at the inhalable fraction.

Currently, there are no standardized protocols to quantify CNT/F structures by electron microscopy, complicating comparisons across studies and limiting the ability to develop meaningful exposure limits. In the interim, the British Standards Institute (BSI) suggested a benchmark exposure limit for fibrous nanomaterials at $0.01 \text{ fibers}/\text{cm}^3$. A fiber is defined as a particle with an aspect ratio $> 3:1$ and length $> 5 \mu\text{m}$ (BSI, 2007), and measurement is by SEM or TEM. About half of the samples collected from participants in this study and analyzed for total TEM concentrations were found to be above the BSI benchmark of $0.01 \text{ fibers}/\text{cm}^3$. However, as indicated from the TEM data of this study, CNT/F materials were typically particle agglomerates from 2 to $10 \mu\text{m}$, and do not fit the traditional definition specified by this benchmark limit. Many of these CNT/F agglomerates will likely fall in the thoracic or inhalable aerosol fractions, based on maximum crosswise dimensions, and are consistent with results from previous studies (Dahm et al., 2015; Kuijpers et al., 2016). This information has great significance as a recent study has indicated that agglomerated CNT/F materials, with particle sizes greater than the respirable upper limit, can deposit in the conducting airways inducing a proliferative airways disease, such as bronchiolitis obliterans (Bishop et al., 2017).

When the particle size distribution data were analyzed at the site level, two facilities (Facility 2 & 12) showed a larger percentage of CNT/F as single fibers or agglomerated particles $< 1 \mu\text{m}$ compared to the remaining facilities. This difference in particle size distribution can likely be attributed to the unique material characteristics at the two facilities. Facility 2 predominantly produced and used CNF, which had the largest average fiber diameter of all the reported materials. The large diameter likely increases the materials' rigidity, limiting its ability to agglomerate (Murray et al., 2012). Similarly, facility 12 had one of the larger reported MWCNT lengths, which may also affect the materials' ability to agglomerate into larger particles. Other factors that may have contributed to this phenomenon are the physical form of the materials (e.g. powder, dispersed liquid), how the materials were handled, and any high-energy tasks performed that may further disperse the materials.

Job titles were standardized based on in-person interviews and observed daily tasks. These standardized titles were designed to be very broad due to the wide variety of job titles and duties at all 12 facilities. Overall differences were seen between Administrators and Technicians at both the respirable and inhalable EC size fractions while differences were also seen between Administrators and Chemists, R&D workers, and Engineers either at the respirable or inhalable size fraction of EC. These results were expected as Technicians and R&D workers commonly handled CNT/F for a majority of the day while job titles such as Engineer and Chemist generally had direct contact with the material $< 50\%$ of their day. Administrators typically did not come into contact with the material, other than incidental exposures. These standardized job titles will form the basis of a predictive model being created to estimate worker exposure using facility and worker level determinants for a job-exposure matrix being developed for analyses of future cohort studies.

To date, only one other study has examined dermal exposure to CNT/F (Hedmer et al., 2015). Although electron microscopy analysis of dermal samples was difficult and time consuming, the results showed

that well over 50% of the participants had evidence of dermal exposures on either their hand or wrist. This raises the possibility of cross-contamination or the settling of suspended CNT/F aerosols in general areas, outside of production or manufacturing areas, which were most notable for Administrators who typically did not directly handle CNT/F.

Additionally, the present study documented internal exposure to CNT/F in sputum, which likely occurred by the exposure routes of inhalation or ingestion. Overall, 18% of the participants had qualitative evidence of exposure by enhanced darkfield imaging, with the highest percentage being found within the secondary manufacturing industry, notably for the R&D workers. Ideally, the presence of CNT/F found within sputum would indicate exposure to aerosols originating from the terminal or bronchiolar airways. However, the quality of each sputum sample was analyzed and most samples contained high counts of oral epithelial cells [$> 80\%$ for all but one participant according to Schubauer-Berigan et al. (in preparation)]. Thus, we cannot rule out the possibility of sputum contamination by exposures in the oral cavity or exposures to larger particles that deposited within the conducting airways of the nasopharyngeal region. Moreover, only a small amount (0.5–1%) of each participant's total sputum sample was analyzed leaving the possibility that CNT/F may have been present in the sample, but not detected. While increasing the survey to a greater percentage of processed sputum would likely increase the incidence.

Furthermore, internal exposures by inhalation and exposures to the hand and wrists were likely dependent on the adherence to and proper usage of PPE, general cleanliness and housekeeping procedures, and other administrative controls which varied from facility-to-facility. Although these two exposure metrics were useful in describing and raising awareness of individual exposures; their overall lack of consistency with the EC and TEM metrics may limit their utility in future epidemiologic exposure-response analyses.

Overall, this study found a similar range of EC mass exposures and size distributions of CNT/F analyzed by electron microscopy as other recently published exposure assessments associated with epidemiologic studies of CNT/F. Previous studies assessed exposures from a single facility and mostly included few participants, limiting their overall generalizability. Lee et al. (2015) conducted a health surveillance study at the largest MWCNT primary manufacturing facility in Korea, which produced $\sim 20 \text{ kg}/\text{day}$. A total of 13 workers participated in the study which included 4 office workers and 9 MWCNT manufacturing workers. Area inhalable samples were collected and analyzed for EC and found to range from $6.2\text{--}9.3 \mu\text{g}/\text{m}^3$; no personal samples were collected from the participants. The samples were also analyzed by SEM, but were not quantified. Visual results from the analysis showed mostly agglomerated MWCNT materials.

A study by Shvedova et al. (2016) and Fatkhutdinova et al. (2016) were conducted as part of an epidemiologic study at a single MWCNT primary manufacturing facility in Russia. A total of 8 exposed workers were examined with an additional 7 controls. The study found 8-h time weighted average personal EC mass exposures at the respirable size fraction to range from $0.66\text{--}2.78 \mu\text{g}/\text{m}^3$ while samples collected at the inhalable size fraction ranged from $3.51\text{--}17.14 \mu\text{g}/\text{m}^3$. Samples were also analyzed by TEM using a modified NMAM 7402, similar to this study and Dahm et al. (2015). The authors primarily found agglomerated materials in the size range of $1\text{--}10 \mu\text{m}$, similar to the present study. However, the TEM structure count results were only reported as a structure per unit area of the filter analyzed and were not extrapolated to an air concentration, limiting their comparability to the TEM results of the present study.

Another exposure assessment in conjunction with a cross-sectional epidemiologic study was conducted by Kuijpers et al. (2016) within a European MWCNT primary manufacturing facility. During MWCNT synthesis a total of $100 \text{ kg}/\text{day}$ could be produced while $\sim 500 \text{ g}$ of material would be used for R&D operations. A total 21 workers and 29 age and gender matched controls participated in the study (Vlaanderen et al., 2017). Inhalable samples collected for the mass concentration of

EC revealed a range of 0.17–1186.52 $\mu\text{g}/\text{m}^3$ and samples analyzed with SEM identified agglomerated materials between 200 nm–10 μm , with a mode diameter between 650 nm and 1 μm .

It has previously been noted that comparing results across investigations can be difficult since measurement strategies and reporting have varied from one study to another (Debia et al., 2016). At the same time, as the harmonization of exposure assessment strategies is still under development for CNT/F, there seems to be a growing consensus among exposure metric selection within published reports. To date, all of the exposure assessments associated with health effects studies have used some combination of size fractionated EC mass sampling along with a visual qualitative or quantitative analysis via electron microscopy. Additionally, many other recently published studies, which solely focused on CNT/F exposure assessments conducted within single facilities, have also assessed exposures using some combination of EC mass and electron microscopy measurements (Birch et al., 2011; Hedmer et al., 2014; Dahm et al., 2015; Fonseca et al., 2015; Thompson et al., 2015; Kouassi et al., 2017).

As many of the epidemiologic data published to date come from small cohorts ascertained from a single facility, an internationally pooled effort will likely be needed to achieve the power necessary to find meaningful results. Therefore, establishing consensus exposure assessment methods and metrics will be critical to this effort, most notably for electron microscopy since no standardized quantitative analysis method specific to CNT/F currently exists.

5. Conclusions

In summary, this is the first multi-site investigation conducted in the US to describe occupational exposures to CNT/F using a multi-metric approach as part of an epidemiologic study of early effect markers. Overall, the data support the appropriateness of combining this multi-metric exposure approach and biological end-point data for further epidemiologic analyses. The study also established heterogeneous levels of exposure which are essential for establishing an exposure-response relationship. Additionally, our results are consistent with several previous international studies and its findings provide the first evidence of internal exposures to CNT/F.

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Competing financial interest declaration

None.

Disclaimer

The findings and conclusions in this manuscript are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health (NIOSH). Mention of any company name or product does not constitute endorsement by NIOSH.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijheh.2018.01.006>.

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