Industrywide Exposure Assessment and Cross-Sectional Epidemiologic Studies of Workers at Facilities Manufacturing, Distributing, or Using Carbon Nanotubes or Carbon Nanofibers in the United States.

Protocol

Project Officers: Mary K. Schubauer-Berigan and Matthew M. Dahm, National Institute for Occupational Safety and Health; Division of Surveillance, Hazard Evaluations and Field Studies; Industrywide Studies Branch

November 5, 2012, revised February 5, 2013

National Institute for Occupational Safety and Health
*Division of Surveillance, Hazard Evaluations and Field Studies; Industrywide Studies Branch
**Division of Applied Research and Technology; Chemical Exposure & Monitoring Branch
Cincinnati, OH
§Health Effects Laboratory Division;
Mention of trade names does not imply endorsement by the United States government or the National Institute for Occupational Safety and Health
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>- Potential health effects of carbon nanotubes and nanofibers</td>
<td>2</td>
</tr>
<tr>
<td>- Potential workplace exposures to carbon nanotubes and nanofibers</td>
<td>4</td>
</tr>
<tr>
<td>BACKGROUND ON CARBON NANOTUBE AND NANOFIBER PRODUCTION AND USE IN THE UNITED STATES</td>
<td>5</td>
</tr>
<tr>
<td>- CNT/CNF primary manufacturing</td>
<td>5</td>
</tr>
<tr>
<td>- CNT/CNF secondary manufacturing</td>
<td>6</td>
</tr>
<tr>
<td>- CNT/CNF distribution</td>
<td>7</td>
</tr>
<tr>
<td>RESULTS OF FEASIBILITY STUDY</td>
<td>7</td>
</tr>
<tr>
<td>- Phase I</td>
<td>8</td>
</tr>
<tr>
<td>- Phase II</td>
<td>11</td>
</tr>
<tr>
<td>STUDY RATIONALE</td>
<td>13</td>
</tr>
<tr>
<td>STUDY OBJECTIVES</td>
<td>13</td>
</tr>
<tr>
<td>METHODS and MATERIALS</td>
<td>14</td>
</tr>
<tr>
<td>I. Industrywide exposure assessment study</td>
<td>15</td>
</tr>
<tr>
<td>Measurement Methods Development</td>
<td>15</td>
</tr>
<tr>
<td>- Exposure Assessment Sampling Strategy</td>
<td>19</td>
</tr>
<tr>
<td>- Sampling Methods</td>
<td>20</td>
</tr>
<tr>
<td>- Information on exposure factors to be collected</td>
<td>24</td>
</tr>
<tr>
<td>- Data analysis methods and assignment of exposures to workers</td>
<td>25</td>
</tr>
<tr>
<td>II. Cross-sectional biomarker study</td>
<td>28</td>
</tr>
<tr>
<td>- Rationale</td>
<td>28</td>
</tr>
<tr>
<td>- Approach</td>
<td>29</td>
</tr>
<tr>
<td>- Biomarkers</td>
<td>30</td>
</tr>
<tr>
<td>- Recruitment of Participants for Exposure Assessment and Epidemiologic Study</td>
<td>36</td>
</tr>
<tr>
<td>- Biomarkers to be analyzed</td>
<td>38</td>
</tr>
<tr>
<td>- Sample Collection and Preparation</td>
<td>39</td>
</tr>
<tr>
<td>- Medical examination methods</td>
<td>45</td>
</tr>
<tr>
<td>- Spirometry methods</td>
<td>45</td>
</tr>
<tr>
<td>- Biospecimen collection methods</td>
<td>50</td>
</tr>
<tr>
<td>- Questionnaire administration</td>
<td>54</td>
</tr>
<tr>
<td>EPIDEMIOLOGIC ANALYSIS</td>
<td>54</td>
</tr>
<tr>
<td>POWER ANALYSIS</td>
<td>56</td>
</tr>
<tr>
<td>HUMAN SUBJECTS PROTECTIONS</td>
<td>62</td>
</tr>
<tr>
<td>- Confidentiality</td>
<td>62</td>
</tr>
<tr>
<td>- Worker Notification</td>
<td>63</td>
</tr>
<tr>
<td>- Study Risks and Benefits</td>
<td>63</td>
</tr>
<tr>
<td>- Assessment of Potential Benefits</td>
<td>63</td>
</tr>
<tr>
<td>- Assessment of Potential Risks</td>
<td>64</td>
</tr>
</tbody>
</table>
Description of Measures Taken to Minimize Potential Risks .................................................. 66
Vulnerable populations ................................................................................................................ 67
Risk versus Benefit Evaluation .................................................................................................. 67
STUDY STRENGTHS AND LIMITATIONS .............................................................................. 68
REFERENCES .......................................................................................................................... 70
APPENDICES: DOCUMENTS PERTAINING TO CROSS-SECTIONAL BIOMARKER STUDY ................................................................. 86
Appendix I. Fact sheet and informed consent documents ........................................................ 87
Appendix II. Questionnaire for participants in study of lung and cardiovascular function, and
biomarkers of early health effects among CNT and CNF workers ...................................... 100
Appendix III: Exposure factors related to company and employee (to be completed by NIOSH
investigator) ................................................................................................................................ 116
Appendix IV. Emergency plan while conducting medical examinations and collecting
biospecimens ................................................................................................................................. 121
Addendum 1: Notification letter #1: immediately available medical results (approved by NIOSH
IRB on 11/29/2012) .................................................................................................................. 122
Addendum 2: Notification letter #2: clinically significant medical findings ............................. 123
Addendum 3: Notification letter #3: Exposure assessment results .......................................... 128
List of Tables

Table 1. Sampling plan to be used for exposure assessment site visits. ........................................ 24
Table 2. Description of circulating biomarkers to be measured for early effect of exposure. ...... 42
Table 3. Panel for blood stimulation study, to be analyzed by NIOSH contractor....................... 43
Table 4. IARC Group 1 carcinogens shown to cause lung cancer, non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), mesothelioma, or nervous system cancer. ................................................................................................................. 44
Table 5. Spirometry safety exclusion or precaution items for adults (age 16-79) (adapted from NHANES 2008). ................................................................................................................................. 46

List of Figures

Figure 1. Universal flow diagram for interpretation of spirometry (adapted by R. McKay from McKay and Horvath 1994). .................................................................................................................. 50
Figure 2. Simulated forced vital capacity (FVC), as percent predicted, resulting from a decrement of 1% per unit of background-corrected elemental carbon exposure, assuming a standard deviation of 10% in FVC among the unexposed......................................................... 59
Figure 3. Effect measure [R2(T)] detectable for forced vital capacity (as a percent of age-, race-, gender- and height-predicted) related to background-corrected elemental carbon at a power of 0.8 and an alpha of 0.05........................................................................................................ 60
Figure 4. Effect measure [R2(T)] detectable for a set of five biomarkers (adjusted for six confounding variables) related to background-corrected elemental carbon at a power of 0.8 and an alpha of 0.05........................................................................................................ 61
### Glossary of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG</td>
<td>8-hydroxy-2’-deoxyguanosine</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>BEV</td>
<td>back-extrapolated volume</td>
</tr>
<tr>
<td>CBC</td>
<td>complete blood count</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>CNF</td>
<td>carbon nanofiber</td>
</tr>
<tr>
<td>CNT</td>
<td>carbon nanotube</td>
</tr>
<tr>
<td>CPC</td>
<td>condensation particle counter</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DART</td>
<td>Division of Applied Research and Technology</td>
</tr>
<tr>
<td>DC</td>
<td>diffusion charger</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSHEFS</td>
<td>Division of Surveillance, Hazard Evaluations, and Field Studies</td>
</tr>
<tr>
<td>DRI</td>
<td>direct-reading instruments</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EC</td>
<td>elemental carbon</td>
</tr>
<tr>
<td>ECN</td>
<td>engineered carbonaceous nanomaterials</td>
</tr>
<tr>
<td>EDXA</td>
<td>energy dispersive X-ray analysis</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
</tr>
<tr>
<td>FEF25-75</td>
<td>forced expiratory flow during the middle two quarters of the FVC</td>
</tr>
<tr>
<td>FEV1</td>
<td>forced expiratory volume in 1 second</td>
</tr>
<tr>
<td>FERV</td>
<td>Field Evaluations and Response Vehicle</td>
</tr>
<tr>
<td>FVC</td>
<td>forced vital capacity</td>
</tr>
<tr>
<td>GC/MS</td>
<td>gas chromatography with mass spectrometry</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>GPx</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>hCAEC</td>
<td>human coronary artery endothelial cells</td>
</tr>
<tr>
<td>HELD</td>
<td>Health Effects Laboratory Division</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule-1</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>inductively coupled plasma with atomic emission spectroscopy</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>IH</td>
<td>industrial hygiene</td>
</tr>
<tr>
<td>KL</td>
<td>Krebs von den Lungen</td>
</tr>
<tr>
<td>LC/MS</td>
<td>liquid chromatography with mass spectrometry</td>
</tr>
<tr>
<td>LOD</td>
<td>limit of detection</td>
</tr>
<tr>
<td>LPM</td>
<td>liters per minute</td>
</tr>
<tr>
<td>LLN</td>
<td>lower limit of normal</td>
</tr>
<tr>
<td>MCE</td>
<td>mixed cellulose ester</td>
</tr>
<tr>
<td>MDC</td>
<td>macrophage derived chemokine</td>
</tr>
<tr>
<td>M-FISH</td>
<td>multiplex fluorescence in situ hybridization</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloprotease</td>
</tr>
<tr>
<td>MW</td>
<td>multi-walled</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NIOSH</td>
<td>National Institute for Occupational Safety and Health</td>
</tr>
<tr>
<td>NMAM</td>
<td>NIOSH manual of analytic methods</td>
</tr>
<tr>
<td>PAH</td>
<td>polycyclic aromatic hydrocarbon</td>
</tr>
<tr>
<td>PAI</td>
<td>plasminogen activator inhibitor</td>
</tr>
<tr>
<td>PBZ</td>
<td>personal breathing zone</td>
</tr>
<tr>
<td>PM</td>
<td>particulate matter</td>
</tr>
<tr>
<td>QFF</td>
<td>quartz fiber filters</td>
</tr>
<tr>
<td>R&amp;D</td>
<td>research and development</td>
</tr>
<tr>
<td>SCGE</td>
<td>single-cell gel electrophoresis</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>SKY</td>
<td>spectral karyotyping</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>SW</td>
<td>single-walled</td>
</tr>
<tr>
<td>t-PA</td>
<td>tissue plasminogen activator</td>
</tr>
</tbody>
</table>
TEM  transmission electron microscopy
TNF  tumor necrosis factor
VCAM  vascular cell adhesion molecule
ABSTRACT

Health effects from exposure to nanomaterials are uncertain, but may be more severe than from larger-sized particles of the same material. This is due to the small size, high surface area per unit mass (i.e., specific surface area) or (in some cases) high aspect ratio of nanomaterials. Carbon nanotubes and nanofibers (CNT and CNF) are among the nanomaterials of greatest interest from a public health perspective because of their potentially asbestiform properties (e.g., high aspect ratio) and toxicological evidence of possible fibrogenic, inflammatory, and clastogenic damage resulting from exposures at occupationally relevant levels. In addition, the useful properties of CNT and CNF have rendered them among the first nanomaterials to be commercially exploited in manufacturing settings. Thus, an epidemiologic study to determine whether early or late health effects occur from occupational exposure to CNT and CNF seems warranted. This protocol describes a cross-sectional study of the small current workforce involved with CNT and CNF in manufacturing and distribution, to be conducted in the following phases: 1) industrywide exposure assessment study to evaluate worker exposure and further development and refinement of measurement methods for CNT and CNF; and 2) a cross-sectional study relating the best metrics of CNT and CNF exposure to markers of early pulmonary or cardiovascular health effects. Future phases described in a forthcoming protocol include the development of an exposure registry that will collect demographic, identifying, work history and (where available) exposure information on workers at companies manufacturing or using CNT or CNF; and a long-term prospective cohort study developed from the registry.
INTRODUCTION

Potential health effects of carbon nanotubes and nanofibers

Human health effects from workplace exposures to engineered nanomaterials are uncertain, as no epidemiological studies have been conducted (Schulte et al. 2009; Laney et al. 2011). However, toxicological studies suggest that certain physical properties of engineered nanomaterials, such as particle number, size, surface area, and shape, may be of greater importance than particle mass (a traditional metric for larger-scale materials) in determining exposure and toxicity. For example, nano-sized carbon particles may be more likely to reach the alveolar region than larger particles and thus may present a greater health hazard. The long, thin shape of carbon nanotubes (CNT) and nanofibers (CNF) may confer asbestiform properties upon elemental carbon (EC) (Seaton and Donaldson 2005; Murphy et al. 2011).

Recent toxicological evidence suggests wide-ranging health effects from exposure to CNT or CNF. These likely include injury at the site of initial exposure [e.g., pulmonary inflammation and subsequent fibrosis, or malignant transformation (Sargent et al. 2009, 2010)] but may also include effects at remote sites (e.g., immunological and cardiovascular effects) due to either translocation of the particles or response to an inflammatory cascade (Seaton and Donaldson 2005, Simeonova and Erdely 2009). In vitro studies have found that CNT and CNF show potentially genotoxic effects, such as mitotic spindle disruption, development of aneuploidy or polyploidy, and formation of micronuclei (Sargent et al. 2009, 2010; Kisin et al. 2011). The toxicity of CNF is not as well studied as that of CNT; however, a recent evaluation of the genotoxicity of CNF (Kisin et al. 2011) found them to be more potent clastogens in the comet
There may be differences in biopersistence, toxicity or potential carcinogenicity by CNT or CNF shape, size, or tendency to agglomerate (Osmond-McLeod et al. 2011). A recent mesothelial instillation study in mice observed that long multi-walled (MW) CNT fibers (~5-50 μm) were retained in the pleura to a greater degree than shorter fibers (Murphy et al. 2011). Our initial feasibility investigations (Dahm et al. 2011; Dahm et al. 2012) suggest that most of the CNT fibers being produced in the U.S. are long, with a mean length of 58.4 μm for CNF, 187.9 μm for single-walled (SW) CNT, and 773.3 μm for multi-walled (MW) CNT (Dahm et al. 2011). These particles also have very high aspect ratios with a strong tendency to agglomerate. However, the agglomerate dimensions (e.g., 1 μm x 3 μm) suggest that they are of inhalable, and most of respirable, size. Furthermore, the extent of de-agglomeration in the lung or bronchial tract is unknown. Questions remain about whether such long fibers are able to penetrate the lung and visceral pleura to reach the parietal pleura and mesothelium. Recent in vitro studies suggest it is the high aspect ratio, not surface area or iron content (a contaminant from the production method employed), of CNT that conveys their highest fibrogenic properties (Sanchez et al. 2011) and likely retention in the pleura (Murphy et al. 2011).

Several national and international organizations such as the National Institute for Occupational Safety and Health (NIOSH), the U.K.’s Health and Safety Executive, the National Cancer Institute, the National Toxicology Program, and the International Agency for Research on Cancer (IARC) have emphasized the need for basic knowledge about the human health effects of exposures to engineered nanomaterials (e.g., IARC 2008). Limitations to the ability to study health effects of CNT exposures in human populations include finding and accessing a large
enough exposed population to study, measuring biologically relevant exposure, the lack of sufficient latency to observe chronic health effects, and understanding which pathways of exposure and effect are most relevant (Schulte et al. 2009; Schubauer-Berigan et al. 2011; Schulte and Trout 2011; Schulte et al. 2011). A recommended approach is to develop an exposure registry that may serve as the sampling frame for a series of biomarker studies, panel studies, or cohorts for long-term epidemiologic follow-up (Laney et al. 2011; Peters et al. 2011; Schulte and Trout 2011).

**Potential workplace exposures to carbon nanotubes and nanofibers**

At present, because of the newness of the technology, much of the occupational exposure to engineered nanomaterials occurs at the research and development (R&D) scale. There have been few reliable surveys of the size of the workforce exposed to nanomaterials. More than 30% of nano-manufacturers worldwide participating in a voluntary survey indicated that they create and handle carbonaceous nanomaterials (e.g., CNT, fullerenes, and carbon black) (Gerritzen et al. 2007). A recent NIOSH feasibility study of companies manufacturing or using engineered carbonaceous nanomaterials in the US (above R&D scale) found that 50 (82%) of 61 were handling CNT or CNF (Schubauer-Berigan et al. 2011). Most of these companies were small, with an average of about 10 workers per company. This workforce (estimated at 500 in 2008) was projected to grow about 16% annually, with CNT use growing more than 20% annually (Schubauer-Berigan et al. 2011). About half the companies identified in the NIOSH study provided information about the size, shape, and other properties of the CNT and CNF produced or used, and about the use of exposure controls and protective equipment for workers (Dahm et al. 2011).
Based on the findings from this feasibility study, in FY10-FY11 NIOSH funded a study to evaluate worker exposures at ten of these 50 CNT and CNF producers and users. As a result of this work (described further below), this protocol describes the development of an exposure registry for workers exposed to CNT and CNF and the conduct of epidemiologic studies designed to evaluate workplace exposures to CNT and CNF. The goal of these studies will be to determine whether exposures to CNT or CNF are related to biomarkers of early health effects or, in the long term, to persistent health effects such as pulmonary fibrosis, cardiovascular disease or cancer.

BACKGROUND ON CARBON NANOTUBE AND NANOFIBER PRODUCTION AND USE IN THE UNITED STATES

Basic information about CNT and CNF manufacturing and use in the U.S. is provided in the following paragraphs. The results of a feasibility investigation regarding exposure assessment and epidemiologic industrywide studies of engineered carbonaceous nanomaterials (ECN) are also described below. Phase I of this investigation describes the enumeration and characterization of ECN manufacturers and users. Phase II considers the exposure characteristics of a group of 10 CNT and CNF manufacturers and users from among the companies participating in Phase I.

CNT/CNF primary manufacturing

Anecdotally, it is apparent that there are a large number of university-based or corporate start-up manufacturers of CNT and CNF, but the size of this workforce is unknown. Beyond pure research-and-development scale, a recent NIOSH feasibility study attempted to identify all U.S. primary manufacturers of these materials (Schubauer-Berigan et al. 2011; Dahm et al. 2011).
the 50 CNT or CNF manufacturers and users, 28 were primary manufacturers. Among these, 65% reported using chemical vapor deposition as a synthesis method, 18% used arc discharge, 12% used flame combustion, and 6% used laser ablation. Synthesis methods are associated with different co-exposures (see next paragraph) as well as potential contact with the CNT and CNF during primary manufacturing. We estimated in our feasibility study that at least 251 workers were involved in primary manufacturing in 2008, some of whom were also involved in secondary manufacturing. Most occurs in the northeastern, Midwestern and western U.S. states, with Massachusetts, California, Ohio and Texas leading the manufacturing efforts.

The primary manufacture of CNT and CNF involves exposure to other potentially hazardous materials, including polycyclic aromatic hydrocarbon (PAHs) formed during synthesis of CNT and CNF, heavy metals used as catalysts, or solvents used in product processing (Birch et al. 2011; Birch 2011).

**CNT/CNF secondary manufacturing**

The recent NIOSH feasibility study (Schubauer-Berigan et al. 2011) found that 32 of the 50 CNT/CNF companies were involved in secondary manufacturing (11 of these were also primary manufacturers). The total workforce size at these companies was estimated at 181, and the companies were located primarily in California, Massachusetts, Ohio and Texas, in descending order. Some companies that are involved in both primary and secondary manufacturing maintain separate facilities (often in different states) for these activities. The most common type of secondary manufacturing includes the production or use of polymers or polymer composite (Khare and Bose 2005), with generally small percentages of CNT and CNF in the mixtures. Other uses include creation and use of CNT or CNF-containing inks for flexible
printing and dispersing or otherwise enhancing the properties of the CNT or CNF materials for other downstream users.

Possible hazards associated with secondary manufacture vary by the type of process, but could include: free CNT and CNF, especially if used in powder form; polymers, carbon fibers or other substances used in polymer or composite production; solvents used in ink generation; adhesives used in fabrication; and dusts generated in the drilling, sanding or machining of parts (Dahm et al. 2012).

**CNT/CNF distribution**

In designing the NIOSH feasibility study, distributors were initially excluded from participation because the focus of the study was on manufacturers (Schubauer-Berigan et al. 2011). However, given the potential for worker exposure in the handling of powder-form CNT and CNF, distributors are considered eligible for the current study. Seven companies (three in California) were identified as distributors in the original database for the feasibility study; since then, several others have been identified as importers and possible distributors. Because the distributors were not included in the original feasibility study, it is unknown how many workers are employed by CNT or CNF distributors.

**RESULTS OF FEASIBILITY STUDY**

As mentioned above, in 2008, NIOSH researchers began an assessment of the feasibility of industrywide exposure and epidemiology studies of workers exposed to ECN (Dahm et al. 2011; Schubauer-Berigan et al. 2011). The purpose was to determine whether industrywide studies are feasible among U.S. workers exposed to ECN, based on workforce size and distribution, exposure levels, sufficient (likely) latency between exposures and possible health
outcomes, and willingness to participate. The feasibility study was conducted in two phases, described further below.

**Phase I**

The goal of Phase I of the feasibility study was to identify the most promising population and ECN type for study, and to consider the most appropriate exposure assessment methods and epidemiologic study design. This phase of the feasibility study identified types of carbon nanomaterials produced; size, shape and quantities manufactured; and facility locations as well as the workforce size (Yencken and Tucker 2009; Dahm et al. 2011; Schubauer-Berigan et al. 2011).

In Phase I, employing industry profiles, internet searches, and personal contacts, we identified 139 potentially eligible companies. To be eligible, companies must be manufacturing (or using in other manufacturing processes) ECN in the U.S. above the research and development scale, or at research and development scale with plans to scale-up within five years. Of these, 61 companies were found to be eligible, and the most common substance manufactured was CNT (by 43 companies). We also found 9 ECN distributors. We enumerated a total of at least 620 workers at the eligible companies, 375 of whom work with CNT. Workforce growth was projected at 15-17% annually across all ECN and was highest for CNT at 22%. Most pilot-scale growth was due to CNT manufacturing. A total of 18,000 kg ECN was estimated to be produced annually in the U.S. at the eligible companies. Many of the companies appeared to be spin-offs using technologies developed at university laboratories. These results are described in Schubauer-Berigan et al. (2011).
Half the manufacturers agreed to complete a questionnaire about their workforce size, materials produced, and the use of controls to prevent occupational exposure (Dahm et al. 2011). Most companies reported using some form of administrative controls, engineering controls, or personal protective equipment to control worker exposure.

From the Phase I feasibility study, the following observations were made that may affect feasibility: 1) Most ECN manufacturers are small companies, often near major universities (i.e., spinoffs of research projects); 2) the largest workforce with greatest scale-up potential at the present time is involved in CNT production, use and distribution; 3) operations involved in handling powdered material are most likely to pose a hazard in the workplace, suggesting that primary and secondary manufacturers and raw material distribution are key for study. As a result, we determined that CNT and CNF manufacturers, powder users, and distributors were most promising for industrywide exposure assessment and epidemiology studies.

At this time, it appears that the potential for CNT and CNF exposure is highest among workers handling forms of nanomaterials that have been shown to cause adverse effects in toxicology studies (e.g., airborne particles or liquid formulations with the potential for dermal exposure). This group includes primary manufacturers, secondary manufacturers (users), and distributors. We recognize that the hazard may extend to workers who use the final products containing CNT and CNF materials. However, there are three main limitations to adding such workers: (1) most primary and secondary manufacturers are currently still in pilot or demonstration scale production and there is not a large enough workforce using or recycling the final products; (2) companies are often reluctant to identify their customers to research investigators; and (3) the forms of the CNT or CNF are often encapsulated into hardened polymers or fixed films and the potential for exposure is likely minimal unless airborne dust is
generated by a work process (e.g. sanding, grinding a composite). And in these cases, the CNT and CNF in the dust is embedded in a polymeric matrix, usually at low mass fraction, which differs from the bulk materials under study.

The efforts recommended as feasible at the end of Phase I were:

1. Conduct an industrywide exposure assessment, developing and deploying the most specific and health-relevant exposure metrics for CNT and CNF.

2. Conduct concomitantly cross-sectional studies using workplace exposure measurements, biomarkers of exposure and of early effects for pulmonary fibrosis, inflammation, cancer or genetic damage, and cardiovascular disease.


4. Consider adding the large research and development enterprises involved in CNT and CNF production.

As mentioned above, based on the Phase I findings and supplemented by Phase II observations while visiting several CNT primary and secondary manufacturers (described further below), we concluded that it was appropriate to add CNT distributors and CNF manufacturers as well. Including CNT and CNF primary and secondary manufacturers as well as distributors, we identified 59 eligible companies with a total of at least 500 workers, for an average of about 10 workers handling or otherwise exposed to CNT or CNF per company. This number differs from the 61 companies mentioned above because the 61 refers to all ECN companies, whereas the 59 companies refers just to those manufacturing, distributing or using CNT or CNF.
Phase II

The goal of Phase II of the feasibility investigation was to estimate occupational exposure levels for workers exposed to the most promising ECNs (on the basis of workforce size and toxicological hazard) identified in Phase I, and to gauge initial interest in participating in an epidemiologic study. In fiscal years 2010 and 2011, walk-through surveys, including task-specific and full-shift personal breathing zone (PBZ) sampling, were conducted at ten CNT and CNF sites, including producers (primary manufacturers) and users (secondary manufacturers). No distributors have as yet agreed to participate. Exposures to MWCNT and SWCNT were evaluated among workers based on EC (Birch et al. 2011) and EC plus transmission electron microscopy (TEM)-based particle counts for air samples (Dahm et al. 2012). Results of the Phase II study (Dahm et al. 2012; Dahm et al., in press) suggest that exposure assessment methodology development should continue concurrently with the industry-wide exposure assessment and epidemiology study to ensure that different metrics of exposure (e.g., mass concentration, particle size distribution, fiber size, shape and agglomeration) can be adequately measured at the low levels at which health effects may occur (Kuempel 2011).

In 2012, a continuation of Phase II is being carried out. Sampling is being conducted at several additional facilities that have agreed to participate in the exposure assessment study. The sampling plan is focused on collecting full-shift PBZ samples for the mass concentration of EC and for analysis by transmission electron microscopy, along with direct-reading instruments (DRI) to collect additional metrics of exposure and to determine how exposures may be occurring (i.e., the emissions source). This information will help establish a worker’s daily exposure compared to the mostly task-based, area samples reported to date.
The Phase II continuation is applying methods used previously to assess exposures as well as field test the suitability of emerging methods for assessing worker exposures to CNTs and CNFs (Evans et al. 2010, Birch et al. 2011, Dahm et al. 2012). Exposure measurement methods work is currently underway to support this and other field studies. Its main objectives include lowering the detection limit for EC (NMAM 5040), a key indicator of exposure to CNTs and CNFs. A higher-flow, PBZ respirable cyclone sampler to assess health-relevant exposures is being evaluated. Other new equipment (such as a personal sampler that measures particle size distribution), which is being designed by NIOSH researchers, will be field tested, if available. Additional work is also underway to determine the best way to conduct microscopy-based, size-specific CNT and CNF structure counts for air samples.

During field investigations, we have observed very high acceptance (and reporting) of use of personal protective equipment (e.g., gloves and laboratory coats) to prevent dermal exposure to CNT and CNF (Dahm et al. 2011, 2012). Despite this, it is uncertain to what extent these measures protect the entire skin surface in regular contact with the nanomaterials: we have frequently observed that wrists are uncovered and may receive exposure to the CNT- or CNF-containing materials. Thus, we will also include an evaluation of exposures to skin surfaces in contact with these materials.

Discussions with plant management and workers in Phase I and II indicate there is an interest from many of the participating companies in also taking part in the epidemiologic component of the study, including a biomarker and prospective cohort study.
STUDY RATIONALE

As described above, numerous in vitro and in vivo toxicology studies have demonstrated the fibrogenic, inflammatory, and possibly mutagenic properties of some CNT and CNF. The feasibility study conducted by NIOSH researchers indicates that there is a growing workforce, currently at least 500 in the U.S., involved in the manufacture, use, and distribution of CNT or CNF. There have been to date no epidemiologic studies published within the workforce exposed to CNT or CNF; however, there is interest in evaluating the potential human health effects of exposure to CNT and CNF. Carbon nanoparticles, primarily SWCNT and MWCNT were identified as a high priority for the evaluation of carcinogenicity by IARC, based on the findings from animal toxicology studies (IARC 2008). As described above, sufficient feasibility now exists to begin conducting industrywide exposure assessment and biomarker studies, and to develop an exposure registry to serve as the framework for a prospective cohort study, which may require international pooling for optimal statistical power.

STUDY OBJECTIVES

The overall study protocol has two major components, summarized below and detailed more extensively in the methods and materials section.

1. Industrywide exposure assessment study: This component will establish sampling and analysis protocols for the detection and quantification of CNT and CNF in US workplaces. Task-based and full-shift exposure assessments, focused on the collection of PBZ samples, will be conducted at facilities participating in the cross-sectional biomarker study and at some other facilities not included in that study. These PBZ samples will be collected for the metrics that are the most current and feasible to assess exposures to CNT or CNF, which are the mass
concentration of EC (a marker for CNT and CNF exposure) as well as size-specific CNT and CNF structure count estimates (Dahm et al. 2012).

**II. Cross-sectional biomarker study:** After the sampling and analysis protocols have been established to measure CNT and CNF, concurrent with the industrywide exposure assessment study, several biomarkers of early effect (for pulmonary fibrosis, cardiovascular disease, and genetic damage) will be measured for workers exposed to a range of CNT and CNF levels. This will be accompanied by a questionnaire to assist in interpretation of the biomarker results and medical examinations to evaluate pulmonary function and cardiovascular health. Statistical analyses will be conducted to determine the nature of the relation between exposure to CNT and CNF and these biomarkers of early effect, considering potential confounding factors such as smoking, age, gender, and workplace co-exposures, including non-engineered particulate matter.

Additional components that are planned for the future include the development of an industrywide exposure registry of CNT and CNF workers, the identification of a cohort of these workers to be followed on a prospective basis for a variety of health outcomes, including incidence and mortality from pulmonary disease (e.g., fibrosis), cardiovascular disease, and cancer. This information will be collected via periodic questionnaires administered to the cohort, and through linkage of the cohort with disease and mortality registries, and consideration of international pooling of cohorts for larger studies to increase statistical power. These additional components are described in a separate protocol that is under development.

**METHODS and MATERIALS**

Methods for the two components of the study are described here.
I. Industrywide exposure assessment study

In this component, we will: 1) refine measurement methods for CNT and CNF, and the metals and polycyclic aromatic hydrocarbons (PAHs) associated with their production (i.e., seen in primary production facilities); 2) conduct task-based and full-shift exposure assessments, focusing on the collection of PBZ air samples; and 3) evaluate dermal exposures on wrists and hands for workers exposed to CNT or CNF. These exposure assessments will primarily consist of samples collected for the mass concentration of EC, a marker for CNT and CNF exposure, and size-specific CNT/CNF structure count estimates from breathing-zone air samples, in a representative group of CNT and CNF manufacturers and users, as well as an assessment of post-shift dermal exposures among these workers.

At least ten companies that have taken part in the Phase I and/or Phase II feasibility study will be invited to participate in this study and the biomarker study, which will be carried out concurrently. The goal is to enroll at least 100 workers, with a range of exposures, in the joint studies. We estimate that 10 employees per company will participate. All facilities included in the study will have been evaluated in Phase II by the field teams in three NIOSH Divisions [the Division of Surveillance, Hazard Evaluations, and Field Studies (DSHEFS), the Division of Applied Research and Technology (DART), or the Education and Information Division (EID)].

Measurement Methods Development

Results of the Phase II feasibility study site visits suggest that exposure assessment methodology development should continue concurrently with the industry-wide exposure assessment study to ensure that different aspects of exposure (e.g., mass concentration, particle
size distribution, fiber size, shape and agglomeration) can be adequately measured at the low
levels at which health effects may occur (Kuempel 2011).

This project will apply methods used previously as well as field-test the suitability of
emerging methods for assessing worker exposures to CNTs and CNFs (Evans et al. 2010; Birch
et al. 2011). As mentioned in the Phase II summary above, the objective for this continued work
is to lower the detection limit for EC (NMAM 5040), which includes evaluating higher-flow,
PBZ respirable cyclones to assess health relevant exposures. Other new equipment (such as a
personal sampler that measures particle size distribution), which is currently being designed by
NIOSH researchers, will be field tested if available and preliminary assessments indicate
suitability for CNT and CNF monitoring.

Modified methods for TEM analysis also will be applied to determine a CNT/CNF
structure count. Specifically, a modified NIOSH Method 7402 will be employed to analyze air
and bulk samples on a JEOL2100F TEM. Modifications mostly entail eliminating steps required
for asbestos identification and classifying and counting CNT/CNF ‘structures’. As with NIOSH
7402, a pump is used to draw a measured volume of air through a polycarbonate or mixed
cellulose ester (MCE) membrane filter. After sample collection, a thin film of carbon is applied
to the filter surface by vacuum evaporation (MCE filters must first be chemically treated to
collapse their pore structure). Three small sections are cut from the carbon-coated filter, placed
on TEM grids, and the filter medium is dissolved by a solvent. This procedure deposits the
carbon film on the grid such that it bridges grid openings and supports the particles in their
original positions on the filter.

After preparation, each grid is examined at low magnification to ensure that the loading
and preparation quality are acceptable for a structure count. Randomly selected grid openings are viewed, the total number of structures and grid openings examined are reported, and representative photomicrographs for each grid are taken. The elemental composition of a nanotube structure is determined by energy dispersive X-ray analysis (EDXA). The structure classification is based on morphology and qualitative EDXA. Ambient outdoor and indoor workplace air samples may contain complex nanotube aggregates (and other particle types). Individual CNT and CNF and more complex particles containing CNT/CNF are counted as ‘structures’, classified as either individual tubes/fibers, clusters or matrices. Along with the air volume, the count may be used to estimate the airborne concentration of CNT/CNF structures.

The method for categorizing structures and determining the accuracy of the count is currently under investigation. Improvements to be evaluated include adding a measurement component to permit size-specific structure counts to be conducted. At present, we are evaluating the classification of CNT structures into five different size-specific ‘bins’ based on the longest dimension. The second dimension is annotated if it is less than half the first dimension. For single nanotubes or nanofibers, mean diameters and lengths are estimated, in addition to counts. Representative photomicrographs are captured and retained in an image library. The MCE filters from each collected sample will be retained to permit the re-evaluation of previously collected samples using the methods determined to be most efficient and accurate at capturing the health-relevant aspects of exposure.

Previous work at a primary CNF manufacturer indicated that workplace exposures also occurred to a complex mixture of CNFs (EC used as a marker for exposure) and production byproducts, which included fine/ultrafine, iron-rich soot (Birch et al. 2011), CO (Evans et al. 2010), and PAHs (Birch 2011). Naphthalene and acenaphthylene were the dominant PAHs in air.
samples. In the raw CNF products, the top three PAHs were pyrene, benzo(g,h,i)perylene, and fluoranthene. Other toxic PAHs also were identified, including the carcinogen benzo(a)pyrene.

As a result of this previous work, screening analyses of bulk samples for PAHs will be conducted as part of this study. We anticipate that PAH and metals samples will be more relevant for primary manufacturers producing CNTs and CNFs as compared to downstream secondary manufacturers using the purified products. The presence of PAH in air and raw (unpurified) CNF samples will be assessed for primary manufacturers, based on professional judgment related to reactor design as well as precursors and catalysts employed. The metal content of CNTs and CNFs also may be a factor in the potential toxicity of these materials. Thus, bulk samples also will be analyzed for residual metals. Contaminants (PAH and metals) in final products of primary manufacturers are expected to be much lower than those in raw products because final products are purified to remove organic and metal impurities.

For PAH analyses, target and non-target analytes will be identified by gas chromatography with mass spectrometry (GC/MS); liquid chromatography with mass spectrometry (LC/MS) may also be applied. Target analytes will be quantified, while non-target analytes will be identified based on a library (MS) search and their reported concentrations will be estimated. Metals will be determined by inductively coupled plasma with atomic emission spectroscopy (ICP-AES) according to NIOSH Method 7300 (with modified sample digestion procedure). Method 7300 includes the following metals: Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sr, Te, Ti, Tl, V, Y, Zn, and Zr. Other techniques (e.g., ICP/MS) also are available for trace metals analyses.
Potential dermal exposures will be assessed in a qualitative fashion. Dermal samples will be collected post-shift for each individual participating in the CNT/CNF exposure assessment sampling. Samples will be collected by using a tape stripping method for the outermost skin layer, the stratum corneum (Rougier et al. 1987; Schneider et al. 2000; Lundgren et al. 2006). Any removed CNTs/CNFs will then be visually identified by scanning electron microscopy (SEM). Specifically, double-sided, adhesive-coated substrate will be used as the sampling medium, which will be analyzed directly by SEM. The tape will be placed with a sticky surface on the skin, with slight pressure being applied. This will provide a qualitative (yes/no) answer to whether CNT/CNF material is present or absent on the sampled area, and possibly a relative loading level (high, medium, low). Two samples will be collected from each worker on their dominant hand. One sample will be collected on the hand, which will include the palm and fingers, and will indicate the effectiveness of gloves and potential hand-to-mouth exposures (ingestion). The other sample will be collected on the wrist and forearm. This sample is representative of the most typical dermal exposure seen in workplaces, between the end of the glove near the wrist and the beginning of the lab coat or Tyvek suit.

**Exposure Assessment Sampling Strategy**

Upon arriving at the facility, NIOSH researchers will hold an initial meeting with company personnel to explain the intentions and needs of the exposure assessment and biomarker study and the site visit as well as preparing the equipment for sampling. A walkthrough of the facility will be conducted to understand possible exposure points and potentially exposed personnel to CNT or CNF.
Filter-based, PBZ samples will be collected for the mass concentration of EC and TEM analysis for each worker in the study (see pp. 36-38 for information concerning worker recruitment). General area samples will be collected at locations deemed to have the highest potential for exposure, based on professional judgment or observations made during any previous NIOSH evaluations of the facility. PBZ samples will be collected throughout the employees’ work shifts. Several DRI will be used alongside the filter-based area samples to assess exposure, non-selectively, to any nano-scale materials. These include a condensation particle counter (CPC 3007; TSI Inc., Shoreview, MN, USA), a photometer (DustTrak DRX Model 8533; TSI Inc.) and a diffusion charger (DC 2000 CE; EcoChem Analytics, Murrieta, CA, USA) (Evans et al. 2010). Task-based area samples will be collected, when feasible, along with concurrent full-shift PBZ samples on the participating employees. These task-based area samples are collected in order to determine any significant tasks that contribute to the worker’s daily exposure and will supplement the total cumulative PBZ samples collected throughout the day.

**Sampling Methods**

Personal and area filter samples will be collected on 25-mm diameter quartz fiber filters (QFF), which will be subsequently analyzed for the airborne mass concentration of EC according to NIOSH Manual of Analytical Methods (NMAM) Method 5040 (NMAM 2006a). Use of these filters facilitates lower detection limits for EC (Dahm et al. 2012). Open-faced cassette sampling will be performed for PBZ and area samples using a Leland Legacy™ (SKC Inc. Eighty Four, PA) pump operating nominally at 6.5 liters per minute (LPM). Respirable PBZ and area samples will also be collected on a 25-mm diameter QFF through use of a special adapter attached to a GK 2.69 BGI cyclone (BGI Inc. Waltham, MA). The cyclone samples will be collected using an XR 5000 (SKC Inc. Eighty Four, PA) pump operating at a flow rate of 4.2 LPM. For area
samples, an additional 37-mm QFF cyclone sample may be collected for comparison to the more-experimental 25-mm QFF cyclone adapter.

During instances of high dust concentrations or heavily contaminated workplaces, all samples will be collected with the respirable cyclones (which operate at lower flow rates) to reduce the likelihood of “overloading” the samples. This will be based upon professional judgment upon arriving at a facility.

Additional personal and area filter samples will be collected on 25-mm mixed cellulose ester (MCE) filters to be analyzed using a modified NMAM 7402 for TEM (NMAM 2006b). XR 5000 or Leland Legacy TM (SKC Inc. Eighty Four, PA) pumps operating at 5 LPM will be used to collect the TEM samples. Depending on the dust levels within the facility, as well as the length of a shift, or the quantities of CNT or CNF used, a 25 mm respirable cyclone may be attached to the 25-mm MCE filter set at a flow rate of 4.2 LPM and used as a personal breathing sample in order to provide a more size-discriminating respirable sample. This will reduce the possibility of filter overloading, which could interfere with the accuracy and quality of TEM analyses. TEM measurements will be used for two main purposes: 1) to provide qualitative evidence of the presence of CNT and CNF in samples; and 2) to facilitate the estimation of size-specific structure counts. These counts, made on a representative number of fields, will be related to the volume of air collected to provide quantitative air concentrations of CNT structures (e.g., Dahm et al. 2012). Typical size, shape and degree of agglomeration will also be denoted using TEM methods. All single fibers or agglomerates containing CNT or CNF, with no size or shape restrictions being used, will be counted and placed into size classes based on length and width, as described above. CNT or CNF agglomerates in open-face filter samples, which may contain non-respirable particles, will also be enumerated with no size restrictions, as described
on p. 16. The size-specific counts will be listed as CNT- or CNF-containing structures per mm² and will then be normalized for air volume and reported as size-specific structures/cm³ of air. All pumps used for EC and TEM measurements will be calibrated before and after each day of sampling.

Real-time measurements of particle number concentration will be performed with a CPC (CPC 3007; TSI Inc., Shoreview, MN, USA). The CPC measures particles in the size range of 10 to 1000 nm. The data output is expressed as total number of particles per cubic centimeter (P/cm³) of sampled air with an upper dynamic range of 100,000 P/cm³.

Real-time respirable mass estimates will be obtained using a photometer (DustTrak Model 8533; TSI Inc). The operating range of the DustTrak is 0.001 to 150 mg/m³, and it measures mass of particles with diameters in the 0.1 to 15 µm size range. Active surface area measurements will be provided by a diffusion charging (DC)-based instrument (DC 2000 CE; EcoChem Analytics, Murrieta, CA, USA). Units are expressed as µm²/cm³, and the DC has an operating range of 0-2000 µm²/cm³. All area direct-reading (real-time) instruments and area filter samples will be placed on a cart approximately 1 m from the ground, to enhance the mobility of the equipment. The DRIs and filter-based air samples will be arranged on the cart with all sampling inlets placed as close as possible to each other in order to sample the same air space. The sampling approaches described have been adapted from Evans et al (2010), Birch et al. (2011) and Methner et al. (2010).

On-board data-logging capabilities will be utilized for the CPC, DustTrak and DC, and the shortest logging intervals will be selected for all samples: 1 second for the indoor CPC and DustTrak and 10 seconds for the DC. Outdoor background samples will be collected using the
CPC and DustTrak, when available, with the logging intervals set at 1 second. Listed below is the sampling plan (Table 1) along with the specific pieces of equipment used to collect all personal and area samples.

Outdoor and indoor background measurements will be collected on each day of sampling due to the potential for contribution of incidental nanoparticles. Anthropogenic sources of fine and ultrafine EC mainly relate to fossil fuel combustion (e.g., motor vehicle exhaust). Sources include diesel engines, emissions from coal-fired and fuel oil-fired power plants, as well as the seasonal burning of biomass (Magliano et al. 1999; Streets et al. 2001; Christoforou et al. 2000; Schauer 2003). In general, nanoscale particles occur as byproducts of combustion or hot processes, such as during operation of motor vehicles, compressors, and industrial dryers and heating systems. Various cleaning operations and condensation processes also can contribute nanoscale particles. The multiple sources of these fine and ultrafine aerosols are significant contributors to the total particle concentration measured by the CPC (Lam et al. 2006, Heitbrink et al. 2007, Demou et al. 2008, Peters et al. 2009, Evans et al. 2010), often precluding reliable detection of engineered nanoparticles over this size range, if present. To account for ambient (environmental and occupational) background, samples will be collected using a CPC (particle number), a DustTrak (particle mass) and filter-based samples for the analysis of the mass concentration of EC and TEM for CNT or CNF structures. The background samples will be collected throughout the full day of sampling. Sample locations for indoor and outdoor background measurements will be selected based on professional judgment and prior knowledge of the facility for those visited previously. In general, however, outdoor background sampling will be used for facilities that are open to outdoor air, and indoor background sampling will be
used for other facilities, at locations that share a common air handling source as the nanomaterial production area, but that have no potential exposure to the CNT or CNF.

### Table 1. Sampling plan to be used for exposure assessment site visits.

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Direct-Reading Instruments</th>
<th>Filter</th>
<th>Cyclone</th>
<th>Nominal Flow Rate (LPM)</th>
<th>Analytical Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal (on each worker)</td>
<td>None</td>
<td>25 mm QFF$^1$</td>
<td>N</td>
<td>6.5</td>
<td>NMAM$^1$ 5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 mm QFF</td>
<td>Y</td>
<td>4.2</td>
<td>NMAM 5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 mm MCE$^1$</td>
<td>N</td>
<td>5</td>
<td>NMAM 7402</td>
</tr>
<tr>
<td>Area</td>
<td>CPC$^1$, DC$^1$, and DustTrak</td>
<td>25 mm QFF</td>
<td>N</td>
<td>6.5</td>
<td>NMAM 5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 mm QFF</td>
<td>Y</td>
<td>4.2</td>
<td>NMAM 5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37 mm QFF</td>
<td>Y</td>
<td>4.2</td>
<td>NMAM 5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 mm MCE</td>
<td>N</td>
<td>5</td>
<td>NMAM 7402</td>
</tr>
<tr>
<td>Outdoor Background</td>
<td>CPC and DustTrak</td>
<td>25 mm QFF</td>
<td>N</td>
<td>6.5</td>
<td>NMAM 5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 mm QFF</td>
<td>Y</td>
<td>4.2</td>
<td>NMAM 5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 mm MCE</td>
<td>N</td>
<td>5</td>
<td>NMAM 7402</td>
</tr>
</tbody>
</table>

$^1$Abbreviations: CPC, condensation particle counter; DC, diffusion charger; LPM, liters per minute; MCE, mixed cellulose ester; NMAM, NIOSH Manual of Analytic Methods; QFF, quartz fiber filter

### Information on exposure factors to be collected

While conducting the site visits, information will be collected on aspects of the workforce, which will permit the evaluation of factors that may affect risk (as confounders or effect modifiers) in epidemiologic studies of CNT and CNF workers. Such information will include number of workers directly (through active use) and incidentally exposed to these materials (in the present and anticipated from near-future scale-up), an evaluation of potentially confounding exposures for pulmonary and cardiovascular diseases, the use of personal protective equipment that might attenuate exposure, and any employer-based medical surveillance of the workforce. A more complete list of the factors to be evaluated includes the following:

- Synthesis method, if a primary manufacturer
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

- Type and toxicity of raw materials and other potential co-exposures
- Nominal aspect ratio of CNT or CNF (as reported by the company and measured in bulk sample whenever possible)
- Type of processes and tasks performed by employees
- Form of CNT and CNF used—dry powder or liquid emulsion
- Use of personal protective equipment
- Length of shift
- Time spent per shift working directly with CNT or CNF
- Time spent per shift potentially indirectly exposed to CNT or CNF
- Cleaning operations and waste disposal
- Workplace medical surveillance

This information will be collected by discussing the work shifts and tasks with each worker, supplemented with direct observations by the study team, evaluation of workplace records, and discussions with the employer. An example data collection form for the field researchers to use in collecting the data is given in Appendix III.

**Data analysis methods and assignment of exposures to workers**

Exposure information will be collected for all workers included in the biomarker study, and the information for these workers will be used as follows: geometric mean exposure estimates from full-shift personal sampling will be developed for EC estimates. Currently, most facilities are only operating for one shift per day but (for facilities with more than one shift), the
number of shifts to be sampled will be decided on a case-by-case basis depending on the number of employees participating and number of shifts being worked.

Background EC concentrations will be subtracted from the occupational EC exposure (but the background exposure will be retained as a potentially confounding exposure). The proportion of the occupational exposure that is derived from CNT or CNF will be estimated based on the EC measurements and the TEM-based, size-specific CNT/CNF structure counts, using methods described in this protocol and in the draft NIOSH Current Intelligence Bulletin for CNT and CNF (NIOSH 2010). The exposure factors mentioned above will be used to modify the measured air concentrations, where appropriate. For example, a worker’s correct application of personal protective equipment may be used to reduce the exposure estimated for the worker for the epidemiologic components of the study.

For EC mass-based measurement data below the limit of detection (LOD), a value of half the LOD will be employed in evaluating exposure-response relations with the cross-sectional study, if the number of non-detectable samples is less than one-third of the total number for all samples in the field study. If the number of non-detectable samples is one-third or more of the total number, then only non-parametric (qualitative) analyses will be used to evaluate the EC exposure metric (e.g., subjects would be classified as “exposed at greater than the detection limit” or “possible exposure below the detection limit”). For microscopy-based samples, a value of zero will be used for less-than-detectable levels (Dahm et al. 2012).

Other exposures co-occurring with the CNT or CNF in each workplace will be summarized using mass concentrations, in the case of PAHs and metals, or as particle counts, for dusts encountered in polymer production or the cutting or sawing of composite materials. The
direct-reading instrument data collected for each task and facility will be interpreted as a non-specific measure of all nano- and ultrafine-sized particles, since particle number and mass concentrations have not been found to be well correlated with CNT or CNF exposures in recent NIOSH evaluations (Dahm et al., in press).

Temporal variability in the exposure measurements will be evaluated. The specificity of each exposure measurement method (DRI, elemental carbon, size-specific structure counts) affects the temporal variability. For example, temporal variability in elemental carbon and DRI measurements is expected due to several seasonal and diurnal factors, such as seasonal or daily peak automobile use and the burning of biomass. These issues will be addressed by collecting indoor or outdoor background samples for the duration of sampling and subtracting the background from the measured full-shift concentration for each worker. By contrast, CNT and CNF structure counts are highly specific, and the potential for detectable background concentrations is minimal.

To address temporal variability in actual CNT or CNF exposures, we will monitor each worker (or, for unexposed or incidentally exposed workers, a single worker representative) for two full shifts during our site visit and will use the average of the CNT structure counts and background-corrected elemental carbon across the two-shift period. Similarly, for DRI (which will be considered exposure to potentially confounding ultrafine particulates) the average of mean exposures from two day-long monitoring periods will be calculated for each worker in the study.

Given the cross-sectional study design, it is not possible to evaluate time-dependent exposures or biomarker results. We will, however, assess the consistency of the exposure
findings for a given set of tasks across two years of monitoring, as each major task at each facility has been monitored for elemental carbon and structure counts by NIOSH in the past (Dahm et al. in preparation; Dahm et al. in press; Birch et al. 2011; Evans et al. 2010; Dahm et al. 2012).

The task-specific area samples will not be used to estimate exposures and link with the health data in the cross-sectional study. Rather, they will be used in the exposure assessment study which is evaluating specific processes or tasks to determine which have the highest potential for exposure. This information is of interest from an exposure assessment and control perspective; moreover, it is likely that the task-specific estimates would be useful in developing job-exposure matrices for future prospective or retrospective cohort studies.

**II. Cross-sectional biomarker study**

**Rationale**

The results of the Phase I and Phase II feasibility investigations indicate that a cross-sectional biomarker study is feasible for the following reasons:

1. In Phase I, we found there is a sufficient workforce size for CNT and CNF facilities (estimated at about 500 in 2008-2009 and growing at an annual rate of 22%). During Phase II investigations in 2010 and 2011 at 12 CNT or CNF facilities, we observed on average about 10 employees per facility who were directly or indirectly potentially exposed to these materials.

2. Phase II found evidence of exposure (i.e., measureable concentrations of EC and CNT or CNF structures) at most of the 12 facilities evaluated in the walk-through survey, and a number were found to be above the draft NIOSH recommended
occupational exposure limit of 7 µg/m³ (Dahm et al. 2012). This exposure level, over a 40-year working lifetime, is estimated to confer a lifetime excess absolute risk of pulmonary fibrosis of 10% (NIOSH 2010; Kuempel 2011).

3. Sufficient exposure variability was observed across the workforce, in either Phase II of this project (Dahm et al 2012) or in previous NIOSH evaluations of CNT and CNF exposure (Birch et al. 2011; Methner et al. 2010; Evans et al. 2010).

4. Evidence from occupational studies of metal-exposed workers (Hamaguchi et al. 2008) or environmental studies of ultrafine particles associated with air pollution (Peters et al. 2011) that pulmonary and cardiovascular effects may be effectively studied using cross-sectional or short-term follow-up studies of biomarkers in smaller numbers of participants.

**Approach**

The cross-sectional study will evaluate pulmonary function and biomarkers related to exposure among workers at companies producing or using CNT or CNF. The biomarkers to be evaluated are related to inflammation, oxidative stress, coagulation, cardiovascular disease, fibrosis, and possibly cancer. We will evaluate each biomarker in whole blood, serum, or plasma, for evaluation of systemic signatures of exposure or effect. In addition, where possible, we will evaluate the same suite of biomarkers in a tissue (preferably, sputum) that is closer to the site of initial exposure (Akpinar-Elci et al. 2005). This will allow us to distinguish localized from systemic responses, an approach that has been recommended for study of nanomaterials (Li and Nel 2011).
A questionnaire will be administered to the study participants (described further below) to allow interpretation of the pulmonary function testing and biomarker analyses. Both the biomarker sampling and questionnaire administration will be conducted concomitantly with the occupational exposure measurements detailed in the Methods and Materials section I above.

**Biomarkers**

*Circulating Biomarkers of Inflammation*

The 19 analytes in the inflammation category (Table 2) are traditional, well-described indicators of pulmonary and systemic inflammation following a toxicant exposure. Specifically, after bolus pulmonary exposure of MWCNT in a murine model, circulating levels of interleukin (IL)-6, CXCL1 (murine analog of IL-8), eotaxin, IL-5, macrophage derived chemokine (MDC/CCL22), and C-reactive protein (CRP) were acutely increased (Erdely et al. 2009a, 2011). These mediators were a direct reflection of the ongoing pulmonary response. Apolipoprotein A-I and A-II, alpha-2-macroglobulin, and complement C3 were increased in the serum 28 days following a single exposure to MWCNT (Erdely et al. 2011). Many of these and other mediators, including IL-1β, IL-6, IL-8, TNFα, CRP, and complement C3, have been implicated in human and animal models of particulate matter exposure (Brook et al. 2010). Other biomarkers chosen for inclusion are IL-18, MDC/CCL22 and granulocyte macrophage colony-stimulating factor (GM-CSF). Similar to IL-1β, IL-18 is a cytokine that is activated by the NALP3 inflammasome. The NALP3 inflammasome is induced by pulmonary injury, including particulate exposures such as silica, asbestos, and CNT (Cassel et al. 2008; Dolinay et al. 2012; Dostert et al. 2008; Hornung et al. 2008; Palomäki et al. 2011; Zhou et al. 2012). Increased IL-18 protein levels measured systemically and in the lung and peripheral blood transcript expression of IL-18 have been observed (Dolinay et al. 2012; Sager et al. 2012; Zhou et al. 2012). The markers
MDC/CCL22 and GM-CSF were elevated in the serum of firefighters soon after the World Trade Center disaster and levels were associated with increased risk of airway obstruction in subsequent years. After CNT exposure, both were induced in the lungs with MDC/CCL22 being detected in the serum (Nolan et al. 2011).

Systemic inflammation can also be assessed by a complete blood count (CBC) with differentials. Increased neutrophil counts following exposure have been found in welders, who are exposed to a variety of ultrafine particles (Kim et al. 2005). Also, increased total white blood cell count and neutrophils were found after a 2 hour exposure to concentrated ambient particles (Brook et al. 2009).

**Circulating Biomarkers of Oxidative Stress**

Oxidative stress has been implicated as a mechanism for pulmonary fibrosis, cancer and cardiovascular disease. Biomarkers that will be evaluated (Table 2) include glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity (Delfino et al. 2011). GPx was altered in welders, and more recently both SOD and GPx activities were related to exposure in a cross-sectional study of nanomaterials workers in Taiwan (Han et al. 2005, Liou 2011). The marker 8-hydroxy-2’-deoxyguanosine (8-OHdG) has been examined in numerous occupations and found to be associated with PAH, volatile organic carbon, and particulate exposure (e.g., Ma et al. 2010, Wang et al. 2011). More recently, 8-OHdG has been evaluated and has been found to be related to CNT exposure (Liou 2011). Similarly, 8-isoprostane, regarded as the gold standard for the assessment of free radical-mediated lipid peroxidation (Delfino et al. 2011), is being evaluated in nanomaterial workers (Liou 2011). Myeloperoxidase is an enzyme that is most abundantly expressed in neutrophils. Circulating myeloperoxidase levels are associated with the
risk of coronary artery disease (Zhang et al. 2001) and increased levels in circulating neutrophils have been shown following pulmonary nanoparticle exposure (Nurkiewicz et al. 2006).

**Circulating Cardiovascular and Coagulation Biomarkers**

Many of the inflammatory and oxidative stress serum cytokines and proteins described above are also relevant to cardiovascular disease (e.g. IL-6, CRP). Additional markers include intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, endothelin-1, tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI)-1, fibrinogen, and von Willebrand factor (Table 2). Changes in PAI-1, t-PA, fibrinogen, and von Willebrand factor were associated with particulate matter exposure (Panasevich et al. 2009, Brook et al. 2010). In addition, PAI-1 was elevated following CNT exposure in a mouse model (Erdely et al. 2009a, 2011). ICAM-1 and VCAM-1 are being utilized in other epidemiological studies and were found to be associated with nanomaterial exposure (Liou 2011). Endothelin-1, a vasoactive peptide, is linked to the progression of atherosclerosis. Increased endothelin-1 levels were found in humans exposed to diesel exhaust (Lund et al. 2009) and in children with chronic PM exposure (Calderón-Garcidueñas et al. 2007).

Evaluation of heart-rate variability was initially considered, as it has been shown to be a potential transient effect of exposure in studies of persons exposed to sources of air pollution [e.g., elevated particulate matter (PM) 2.5] (Davoodi et al. 2010; Link and Dockery 2010; Weichenthal et al. 2011). Heart-rate variability has also been recently found to be correlated with PM-2.5 from air pollution (Baccarelli et al. 2008), welding fume exposure (Fang et al. 2008), and urinary biomarkers of PAH exposure (Lee et al. 2011). However, a recent randomized trial with transient diesel exposure showed no association with heart-rate variability (Mills et al. 2011).
The facilities included in the feasibility and exposure assessment study also feature wide variability in background and process-associated ultrafine particulate exposure (Dahm et al., in press). Given the substantial uncertainty in interpreting heart-rate variability data for the relatively low CNT and CNF exposure in the U.S. workforce against a backdrop of highly variable (and often high) ultrafine particulate exposure (e.g., Evans et al. 2010), this measure will not be included in the present study at this time. A static measure of heart rate will be collected, however, as part of the overall basic medical examination.

**Circulating Biomarkers of Pulmonary Fibrosis, Genetic Damage, or Cancer**

Serum Krebs von den Lungen (KL)-6 glycoprotein has been found to be correlated to extent of pulmonary fibrosis and inflammation (Yokoyama et al. 1998; Ichiyasu et al. 2012). This biomarker has also been evaluated in workplaces with potentially hazardous exposures: KL-6 has been used as a marker of early pulmonary fibrosis in studies of workers exposed to indium or cobalt-tungsten carbide (Chonan et al. 2007; Hamaguchi et al. 2008; Kaneko et al. 2010). The matrix metalloproteinases (MMPs) and their inhibitors, e.g., tissue inhibitor of metalloproteinase (TIMP), are well documented to be involved in fibrosis with the potential for systemic evaluation after particle exposure (Erdely et al. 2009a; Rosas et al. 2008, Lund et al. 2009). Osteopontin has been suggested as a biomarker for asbestos-exposed individuals (Park et al. 2009). MWCNT exposure was found to significantly increase pulmonary protein expression of osteopontin (Erdely et al. 2009b). Serum levels of osteopontin were generally increased in those mice exposed to MWCNT although the results were not always consistent. This may be the result of a small sample size (Park et al. 2009) and the artificial nature of exposure conditions.
Biomarkers of early potential carcinogenic effect will be evaluated in this study. Several serum cytokines [e.g., IL-6, IL-8 and C-reactive protein (CRP)] have been shown to be associated with lung cancer incidence (Brichory et al. 2001; Il’yasova et al. 2005; Kaminska et al. 2006; Siemes et al. 2006; Heikkila et al. 2007; Allin et al. 2009; Pine et al. 2011). Pine et al. (2011) found the latter two markers in combination to show the most robust association with lung cancer several years in advance of diagnosis. Based on findings of interference of CNT and CNF with mitotic spindle fiber formation and chromosomal aberrations in animal studies (Sargent et al. 2009; Kisin et al. 2011), we will collect biospecimens (serum, nasal and sputum or buccal cells) to be banked for future measurement of biomarkers of gross chromosomal aberrations. The samples will be banked to permit their analyses, pending available future funding, after all sites are visited and all specimen collection is completed.

The markers of DNA damage and impaired repair mechanisms to be analyzed include single-cell gel electrophoresis (SCGE; comet assay) and multiplex fluorescence in situ hybridization (M-FISH). SCGE has been used as a measure of strand break rejoining in peripheral blood lymphocytes, where it was found to be associated with whole-blood chromium(VI) in electroplating workers (Zhang et al. 2011). M-FISH has been found to be associated with low-dose ionizing radiation exposure in studies of radiological technologists (Sigurdson et al. 2008) and airline pilots (Yong et al. 2009) and has been evaluated in persons exposed to radiation from the Chernobyl disaster (Hieber et al. 2011).

Additional Studies to determine biological effects of CNT or CNF exposure in workers

Leukocyte profiling is being increasingly used in research related to development of a diagnostic tool (Dolinay et al. 2012; Scherzer et al. 2007). Following silica exposure in rats, a
specific blood gene expression pattern was observed that was a surrogate for pulmonary toxicity (Sellamuthu et al. 2011). Previous work has shown that CNT exposure is able to induce leukocyte activation as determined by whole blood gene expression changes (Erdely et al. 2009b). Ongoing inhalation studies in animals exposed to MWCNT are exploring the possibility of a molecular signature that will indicate exposure. Because of the potential applications of expression profiling in leukocytes, whole blood will be collected and RNA will be isolated and banked. Further analysis will depend on available funding and results from the animal studies.

In conjunction with the circulating biomarkers measured above, we hypothesize that, among workers exposed to CNT or CNF, the ability for circulating leukocytes to respond to a secondary stimulation may be compromised. An animal model testing this hypothesis showed decreased responsiveness of circulating leukocytes following metal-rich particulate matter exposure (Hulderman et al. 2012). These results were observed without significant changes in circulating inflammatory cytokine levels, indicating that some circulating markers lack sensitivity, or that a secondary challenge may be needed for decreased responsiveness to be evinced. The human leukocyte response to chronic CNT or CNF exposure may alter cytokine and chemokine production, suggesting the potential for immune dysfunction and chronic inflammation. To evaluate this hypothesis, whole blood cells for workers in the study will be incubated in a null (no stimulant) and stimulant tube for 24 hours, followed by supernatant collection. The analysis will measure the changes in 46 analytes (Table 3), the contractor’s specific panel for this technology. Since the analyte measurements are made after ex vivo manipulation, the changes in analyte values by themselves do not have any clinical relevance for this study. These analytes are chosen simply as responsive proteins that are likely to change following stimulation. The change in these proteins (null vs. stimulated) will be compared to
level of exposure and therefore serve as a potential biomarker of exposure. This approach will add power to the cross-sectional design of this study in that each individual will serve as their own baseline (null tube), eliminating a major source of variability among individuals.

Additional biomarker evaluation will be performed using a translational in vitro model (Channell et al. 2012). The methodological design tests the capacity of the collected plasma (or serum) from workers enrolled in the study to activate primary human coronary artery endothelial cells (hCAEC). In a controlled human exposure to diesel, plasma from exposed individuals resulted in increased cardiovascular disease related markers produced by hCAEC (Channell et al. 2012). The changes in these mediators in the cell line serve as evidence of circulating factors in the plasma (or serum) of exposed workers capable of inducing endothelial activation. This design will be useful in determining potential effects, in addition to those described above in the section on circulating coagulation and cardiovascular biomarkers, on the cardiovascular system of workers exposed to CNT or CNF.

**Recruitment of Participants for Exposure Assessment and Epidemiologic Study**

The source population consists of facilities producing, using or distributing CNT or CNF at above R&D scale in the U.S. The population was enumerated in the Phase I feasibility study and has been augmented since then during Phase II using several additional sources (e.g., registrations with the US Environmental Protection Agency to manufacture or use CNT or CNF). There are currently at least 66 eligible companies. A sample size of at least 100 workers (based on the results of power analyses) will be sought. Given the small average company size, at least 10 companies will need to be included in the study to achieve this number. As described in Methods and Materials Section I above, these will be selected from among the companies...
included in the exposure assessments conducted in FY2010 through FY2012, or previously visited by the NIOSH EID or DART field teams. The criteria for selection of a facility for an invitation to join the study will be:

1) Potential for elevated (near or above the current NIOSH draft REL of 7 µg/m³ as EC) CNT and CNF exposure (based on previous NIOSH site visits);

2) Number of workers routinely exposed, directly and indirectly, to CNT or CNF;

3) Representativeness of site activities across the spectrum of primary and secondary manufacturing and distribution of CNT and CNF;

4) Lack of substantial exposure potential to other agents (not part of the manufacturing process) that may cause adverse pulmonary effects, such as cancer (e.g., some of the substances in Table 4). For example, facilities with very high indoor background concentrations of total particulates due to proximity to a busy highway may receive lower priority for inclusion.

Eligible companies will be ranked according to these criteria and will be invited to participate in the order of highest to lowest priority. NIOSH project staff will travel with the Field Evaluations and Response Vehicle (FERV) to each location. The FERV provides a clean, private space for conducting questionnaire administration, medical examinations, spirometry, sputum collection or induction, and biospecimen collection.

The workforce involved in CNT and CNF production and use is still relatively small. Power calculations conducted for forced vital capacity (a measure of possible fibrotic lung changes) and biomarker measurements suggest that 100 study subjects will be required to observe possible impacts suggested from other studies (see “Power Analysis” section). Because
statistical power will be maximized through the use of regression techniques (rather than comparison of an “exposed” to a “control” group), it is necessary to include workers who have minimal or no exposure as well as workers who have regular exposure to CNT or CNF. Therefore, within each company, all workers will be invited to participate in the cross-sectional study, which involves both biomarker and exposure assessments, and will be given a fact sheet about the study (Appendix I). Workers may decline to participate in any aspect of the study, without jeopardizing their ability to participate in the remaining aspects of the study. For each participating facility, workers agreeing to participate will be administered an informed consent document while NIOSH researchers are on-site to carry out the study (see Appendix I) prior to measuring exposure, collecting any biospecimens, conducting physical examinations, or administering the questionnaire. The lead project officers will be available at all times that the study is being conducted to answer questions from workers or company representatives about the study.

**Biomarkers to be analyzed**

The biomarkers of exposure or early effects to be measured, the biomarker target, and the specimen matrix are detailed in Table 2. The rationale for inclusion of these biomarkers is described above. Most biomarker analyses will be conducted by a contract laboratory, using existing contracts at NIOSH-HELD, NIOSH-DSHEFS, or NIOSH-DART. A few of the biomarker analyses (IL-18, SOD activity, GPx, KL-6, 8-isoprostanetetra, 8-OHdG) will be conducted by NIOSH-HELD.
Sample Collection and Preparation

Given the nature of tests to be done, a specific protocol and order for the blood draw tubes for biomarker analysis is necessary. A total of 44 mL will be collected. The order of the tubes for the blood draw will be as follows:

1) Serum separator tubes (2) – 2 tubes of 5 mL each (for serum to be sent to NIOSH-HELD)

2) Lithium heparin (1) – 4 mL (whole blood collection for ex vivo stimulation. Collected supernatants will be frozen and sent to NIOSH-HELD. A contract laboratory will analyze for a specific analyte panel indicated in Table 3)

3) EDTA (3) – 2 tubes of 8.5 mL each (for plasma to be sent to NIOSH-HELD and contract laboratory), 1 tube of 3 mL (whole blood for CBC at reference lab)

4) PAXgene (4) – four tubes of 2.5 mL each (whole blood collected into a stabilization agent, frozen, and sent to NIOSH-HELD for RNA isolation)

Samples will be treated in the following manner. Serum tubes will be inverted 5 times and allowed 30 min to clot. Plasma tubes will be inverted 10 times and immediately spun down. The immediate spin is recommended for oxidative stress biomarkers. All serum and plasma samples to be fractionated will be spun at 1000 g for 10 min at 4ºC then aliquoted and frozen. The 3 mL EDTA tube of whole blood for the reference lab will be inverted 10 times and stored at room temperature until sent for analysis at the end of the day. The lithium heparin tubes will be inverted 10 times and 1 mL of whole blood will be transferred to each TruCulture stimulation tube (1 null and 1 stimulant tube) immediately after the blood draw. The TruCulture tubes are inverted to mix and then incubated at 37ºC for 24 hours. At 24 hours, the supernatants will be
collected, frozen at -20 °C, and banked at NIOSH-HELD for further analysis. The PAXgene tubes are drawn last due to the stabilization agent contained within the tubes. The tubes are inverted 8-10 times after the blood draw and then placed at -20°C. All frozen samples will be sent overnight on dry ice to NIOSH-HELD pending further analysis.

Sputum specimens will be treated with a mucolytic agent [Sputolysin® or dithiothreitol (DTT)] and incubated at 15 minutes in a shaking water bath set at 37°C, centrifuged at approximately 500 x g, and separated into an acellular and cellular fraction within one hour of collection. The acellular fraction will be frozen at -40°C in the field and shipped on dry ice to the HELD laboratories, where it will be analyzed along with the associated serum specimens by either HELD or the contract laboratory (Table 2).

The cellular fraction will be used for future FISH analyses (pending available funding), and to examine using dark-field microscopy for evidence of CNT or CNF in the cellular matrix. Sputum quality will be evaluated (at NIOSH or by a contractor) by counting the proportion of total cells that are squamous epithelial cells, using <80% as an evaluation point. The cellular pellet will be resuspended in an alcohol-based fixative/cryoprotectant (Saccomanno fluid or methanol) prior to shipment on ice to a HELD laboratory (at least 50% preservative concentration). After arrival, a cytospin of the cell pellet is 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. For analysis by FISH and dark-field microscopy, approximately 3,000 cells per slide will be viewed. As typical sputum samples contain approximately 1 million cells, only a small fraction of the sample is necessary for the FISH and CNT/CNF microscopic analysis. A cytospin of the acellular fraction will also be prepared and examined for potential CNT or CNF in the sputum matrix outside the cells.
To enhance the contrast of nanomaterials for dark-field microscopy, cytospin slides are stained with Sirius Red. Sirius Red staining consists of immersion of the slides in 0.1% Picrosirius solution (100 mg of Sirius Red F3BA in 100 ml of saturated aqueous picric acid, (pH 2) for 1 hour followed by washing for 1 minute in 0.01 N HCl. Sections are then briefly counterstained in freshly filtered Mayer’s hematoxylin for 2 minutes, dehydrated, and coverslipped.
Table 2. Description of circulating biomarkers to be measured for early effect of exposure†.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sample Matrix</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-1β‡</td>
<td>Plasma &amp; Sputum</td>
<td>These 19 analytes are markers of inflammation. As a group, the analytes chosen represent a thorough early screen of effect for exposure to CNT/CNF. These markers have been shown to be increased in animal models of CNT exposure or associated pulmonary exposure studies.</td>
</tr>
<tr>
<td>Interleukin-2§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-4§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-5§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-6§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-8§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-10§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-12p70†</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-18*</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Interleukin-6 receptor beta§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Alpha-2-Macroglobulin§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Complement C3§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>C-Reactive Protein§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>TNFa§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>GM-CSF§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Macrophage derived chemokine§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Eotaxin-1§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A-I§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A-II§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>CBC with Differential§</td>
<td>Whole Blood</td>
<td>Increased neutrophils following concentrated ambient particles or welding fume exposure.</td>
</tr>
<tr>
<td><strong>Oxidative stress</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloperoxidase§</td>
<td>Plasma &amp; Sputum</td>
<td>These markers will indicate the presence of local and systemic oxidative stress. These markers have been indicated following pulmonary toxicant exposures and/or are being analyzed in other nanomaterial epidemiological studies.</td>
</tr>
<tr>
<td>SOD activity*</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>GPx activity*</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>8-OHdG*</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>8-isoprostane*</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular / Coagulation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1§</td>
<td>Plasma &amp; Sputum</td>
<td>These markers represent a group of cardiovascular and coagulation specific markers. The analytes have been increased following pulmonary inflammatory exposures.</td>
</tr>
<tr>
<td>VCAM-1§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Endothelin-1§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>von Willebrand Factor§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>PAI-1§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>t-PA§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td><strong>Cancer / Fibrosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KL-6*</td>
<td>Serum &amp; Sputum</td>
<td>These analytes represent markers of fibrosis and/or cancer. KL-6 and MMPs correlate with pulmonary fibrosis. Some can be increased in incidences of lung cancer.</td>
</tr>
<tr>
<td>MMP-1§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>MMP-2§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>MMP-7§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>MMP-9§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>TIMP1§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td>Osteopontin§</td>
<td>Plasma &amp; Sputum</td>
<td></td>
</tr>
<tr>
<td><strong>Genetic Damage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comet Assay*‡</td>
<td>Serum and either sputum, nasal or buccal cells</td>
<td>Marker of DNA strand breaks and misrepairs; found to be elevated in Taiwanese CNT workers.</td>
</tr>
<tr>
<td>M-FISH*‡</td>
<td>Serum and either sputum, nasal or buccal cells</td>
<td>Marker of chromosome translocations</td>
</tr>
</tbody>
</table>

‡ Analysis to be conducted by NIOSH contractor  
* Analysis to be conducted by NIOSH-HELD  
¶ Future funding  
† All except GPx and SOD expected to increase with CNT or CNF exposure. GPx and SOD expected to decrease.
Table 3. Panel for blood stimulation study, to be analyzed by NIOSH contractor.

<table>
<thead>
<tr>
<th>Alpha-1-Antitrypsin</th>
<th>Alpha-2-Macroglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-2-Microglobulin</td>
<td>Brain-Derived Neurotrophic Factor</td>
</tr>
<tr>
<td>Complement C3</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>Eotaxin-1</td>
<td>Factor VII</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>Granulocyte-Macrophage Colony-Stimulating Factor</td>
<td>Haptoglobin</td>
</tr>
<tr>
<td>Intercellular Adhesion Molecule 1</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>Interleukin-1 alpha</td>
<td>Interleukin-1 beta</td>
</tr>
<tr>
<td>Interleukin-1 receptor antagonist</td>
<td>Interleukin-10</td>
</tr>
<tr>
<td>Interleukin-12 Subunit p40</td>
<td>Interleukin-12 Subunit p70</td>
</tr>
<tr>
<td>Interleukin-15</td>
<td>Interleukin-17</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>Interleukin-23</td>
</tr>
<tr>
<td>Interleukin-3</td>
<td>Interleukin-4</td>
</tr>
<tr>
<td>Interleukin-5</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>Interleukin-7</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>Macrophage Inflammatory Protein-1 alpha</td>
<td>Macrophage Inflammatory Protein-1 beta</td>
</tr>
<tr>
<td>Matrix Metalloproteinase-2</td>
<td>Matrix Metalloproteinase-3</td>
</tr>
<tr>
<td>Matrix Metalloproteinase-9</td>
<td>Monocyte Chemotactic Protein 1</td>
</tr>
<tr>
<td>Stem Cell Factor</td>
<td>T-Cell-Specific Protein RANTES</td>
</tr>
<tr>
<td>Tissue Inhibitor of Metalloproteinases 1</td>
<td>Tumor Necrosis Factor alpha</td>
</tr>
<tr>
<td>Tumor Necrosis Factor beta</td>
<td>Tumor Necrosis factor receptor 2</td>
</tr>
<tr>
<td>Vascular Cell Adhesion Molecule-1</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>Vitamin D-Binding Protein</td>
<td>von Willebrand Factor</td>
</tr>
</tbody>
</table>
Table 4. IARC Group 1 carcinogens shown to cause lung cancer, non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), mesothelioma, or nervous system cancer.

<table>
<thead>
<tr>
<th>Group 1 IARC carcinogen</th>
<th>Site for which evidence is sufficient or limited</th>
<th>Likelihood of exposure in CNT/CNF industry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha particle emitters</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Aluminum production</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Lung, mesothelioma</td>
<td>Low</td>
</tr>
<tr>
<td>Benzene</td>
<td>CLL, NHL</td>
<td>Low</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>None*</td>
<td>High</td>
</tr>
<tr>
<td>Beryllium</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Bis(chloromethyl)ether/chloromethyl methylether</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>1,3-Butadiene</td>
<td>CLL, NHL</td>
<td>Low</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Lung</td>
<td>Moderate</td>
</tr>
<tr>
<td>Chromium(VI)</td>
<td>Lung</td>
<td>Moderate</td>
</tr>
<tr>
<td>Coal gasification, coke production, coal tar pitches</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Diesel exhaust</td>
<td>Lung</td>
<td>Moderate</td>
</tr>
<tr>
<td>Dioxins &amp; furans</td>
<td>Lung, NHL</td>
<td>Low</td>
</tr>
<tr>
<td>Erionite</td>
<td>Mesothelioma</td>
<td>Low</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>CLL, NHL</td>
<td>Low</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>CLL</td>
<td>Low</td>
</tr>
<tr>
<td>Iron &amp; steel founding</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Nickel (non-metallic)</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Occupation as a painter</td>
<td>Lung, mesothelioma</td>
<td>Low</td>
</tr>
<tr>
<td>Rubber manufacturing industry</td>
<td>CLL, lung, NHL</td>
<td>Low</td>
</tr>
<tr>
<td>Silica (crystalline)</td>
<td>Lung</td>
<td>Moderate</td>
</tr>
<tr>
<td>Soot</td>
<td>Lung</td>
<td>High</td>
</tr>
<tr>
<td>Sulfur mustard</td>
<td>Lung</td>
<td>Low</td>
</tr>
<tr>
<td>Strong inorganic acid mists</td>
<td>Lung</td>
<td>Moderate</td>
</tr>
<tr>
<td>Talc</td>
<td>Lung, mesothelioma</td>
<td>Low</td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td>Lung</td>
<td>High</td>
</tr>
<tr>
<td>X- or γ-radiation</td>
<td>Lung, nervous system</td>
<td>Low</td>
</tr>
</tbody>
</table>

*IARC designation is based on mechanistic information alone
Medical examination methods

After obtaining informed consent, the medical examinations to be carried out for each participant in the cross-sectional study include the following:

1. Administration of health questionnaire (see information below)
2. Measurement of height and weight [to interpret spirometry testing and provide accurate body mass index (BMI)]
3. Waist circumference (to use as a possible indictor of metabolic syndrome, which may be useful in interpreting cardiovascular disease biomarkers).
4. Measurement of heart rate and blood pressure (systolic and diastolic individually).
5. Pulmonary function testing via spirometry
6. Collection of biospecimens for biomarker analyses (see information below)

Medical and epidemiological professionals with NIOSH will conduct medical examinations (physician), administer the questionnaire, carry out the pulmonary function testing using spirometry and biological specimen sampling (blood, as well as sputum, nasal or buccal cells), as described below.

Spirometry methods

Spirometry will be conducted using procedures outlined by the American Thoracic Society (ATS)-European Respiratory Society (ERS) (Miller et al. 2005). All spirometry examinations will be conducted by technicians who have passed a NIOSH-approved spirometry training course, have had several hundred hours of experience in conducting spirometry testing, and will be overseen by a Principal Investigator with specialized training in the interpretation of spirometry data.
Before beginning spirometry examination, each subject will be asked about medical conditions that might be contraindications for use of spirometry, taken from the most recent survey conducted by the National Health and Nutrition Examination Survey (NHANES) (Table 5). Inquiry will also be made of medical conditions that lead to precautions in the use of spirometry (Table 5). Questions about chest, abdominal, oral and facial pain will be asked by the spirometry technician just before beginning the procedure to ensure that the proper precautions may be taken during the test. Age, race, and sex will be asked of each subject, and height will be measured (with the subject in stocking feet) to the nearest half-centimeter, using a stadiometer. Questions about cigarette smoking will also be included, to assist in interpretation of spirometry patterns.

Table 5. Spirometry safety exclusion or precaution items for adults (age 16-79) (adapted from NHANES 2008).

<table>
<thead>
<tr>
<th>Screening item</th>
<th>Screening level</th>
<th>Precaution advised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye surgery in the last 3 months</td>
<td>Exclusion</td>
<td>Exclusion</td>
</tr>
<tr>
<td>Chest or abdominal surgery in last 3 months</td>
<td>Exclusion</td>
<td>Exclusion</td>
</tr>
<tr>
<td>Self or household member tuberculosis exposure</td>
<td>Exclusion</td>
<td>Exclusion</td>
</tr>
<tr>
<td>History of aneurysm or collapsed lung</td>
<td>Exclusion</td>
<td>Exclusion</td>
</tr>
<tr>
<td>History of detached retina</td>
<td>Exclusion</td>
<td>Exclusion</td>
</tr>
<tr>
<td>Heart attack or stroke within the past 3 months</td>
<td>Exclusion</td>
<td>Exclusion</td>
</tr>
<tr>
<td>Presence of respiratory infection</td>
<td>Precaution</td>
<td>Test at end of day; decontaminate tubing and use gloves</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Precaution</td>
<td>Test at beginning of day to minimize chance of infection</td>
</tr>
<tr>
<td>Chest or abdominal pain of any cause</td>
<td>Precaution</td>
<td>Monitor carefully to ensure test not too demanding</td>
</tr>
<tr>
<td>Oral or facial pain exacerbated by mouthpiece</td>
<td>Precaution</td>
<td>Use alternative mouthpiece shapes</td>
</tr>
</tbody>
</table>
Spirometry methodology will be conducted using the steps outlined in the procedures document for the NHANES survey (NHANES 2008). In brief summary, a volume-based spirometer will be used for all spirometry testing (OMI/Sensormedics 1022), in order to maximize measurement accuracy (expected to be better than 1.5%). Diligent and consistent coaching will be used by the spirometry technician to ensure maximum inspiration and forced expiration during each test.

Standard acceptability criteria for satisfactory start and end of spirometry tests will be used (Miller et al. 2005). More specifically, an acceptable test start must not involve excessive hesitation (evaluated as a BEV ≥5% of FVC or greater than 150 mL) or cough in the first second. In addition, a rise time to peak flow of <120 msec and volume at peak flow at <35% of FVC, will be required. The end-of-test criteria to be used are either a) the subject cannot or should not continue further exhalation (due to discomfort or the appearance of approaching syncope) or b) the volume-time curve shows a change-in-volume of less than 0.025 L for at least 1 second and the subject has tried to exhale for at least 6 seconds (i.e., an acceptable plateau was reached, as determined by the spirometry software). Tracings that do not meet acceptability criteria but that do provide usable information will be retained and used in relevant analyses.

At least three (and up to eight) spirometry maneuvers will be conducted per subject, based on three tests meeting criteria for acceptable start and end of test, and acceptable repeatability among the FVC and FEV$_1$. The technician will employ the ATS-ERS criteria (Miller et al. 2005) for repeatability: the two highest usable FVCs, and the two highest usable FEV$_1$s will each differ by less than 0.15 L. Although not required by ATS-ERS criteria, a peak flow that differs by no more than 20% between the two highest values will be used as an additional measure of repeatability.
Software to be used will comply with recommendations of the ATS-ERS (Miller et al. 2005), and will use sex-, height-, and race-specific predictive values for lung function metrics such as FEV$_1$ and FVC (Hankinson et al. 1999). Quality control checks to be used to test accuracy of volume, lack of leaks, volume linearity, time, and other variables will be in accordance with ATS-ERS recommendations for volume spirometers (Table 3 of Miller et al. 2005).

An anti-contamination plan will be used, consisting of the following:

1. Use of single-use mouthpieces with a high-quality bacterial and viral filter
2. Single-use nose clips
3. Flushing of spirometer with room air between subjects
4. Rotation of spirometer tubing to keep dry
5. Disinfection of tubing between subjects
6. Waiting period of at least one-half hour between subjects
7. Washing hands between subjects
8. Avoiding contact with contaminated surfaces

Spirometry software will be used to calculate relevant parameters, including back-extrapolated volume (BEV), forced expiratory volume in the first second (FEV$_1$) and forced vital capacity (FVC), percent predicted, FEV$_1$/FVC% (using the largest valid FEV$_1$ and FVC, even if from different tests). All values will be corrected for pressure differences at body compared to ambient temperature. The interpretation software module developed by McKay will be used to assist in measuring compliance of the test performance with ATS-ERS standards, and in providing interpretations of observed spirometry patterns. Values will be compared to the “lower limit of normal”, based on age-, height-, sex- and (for white, African-American, and Hispanic subjects) race/ethnicity-specific NHANES population data from Hankinson et al. (1999). For
subjects of East Asian descent, an adjustment factor of 0.94 based on white subjects will be used, in accordance with ATS-ERS recommendations, and for those of South Asian descent, the value for white subjects will be used unadjusted (Miller et al. 2005; Hankinson et al. 2010; R. McKay, University of Cincinnati, personal communication).

For the research study, lung function patterns resulting from spirometry will be interpreted using a universal flow diagram, as developed by McKay and Horvath (1994) (Fig. 1). Restrictive lung patterns will be identified as a FEV\textsubscript{1}/FVC above the lower limit of normal (LLN; the lower one-sided confidence limit on the predicted age, sex, race/ethnicity, and height-adjusted value), but with a LLN for FVC below the LLN. Obstructive lung patterns will be identified as an FEV\textsubscript{1}/FVC below the LLN, an FVC above the LLN, and the FEV\textsubscript{1} \textless 95% of predicted (the latter accounts for some thin, healthy persons with large FVC but normal FEV\textsubscript{1}). The FEF\textsubscript{25-75} (obtained from the volume-time tracing of the curve that gives the largest sum of the FEV\textsubscript{1} and FVC) that is below the LLN will be used to as a more sensitive measure of possible early obstructive lung patterns, among those with otherwise normal FEV\textsubscript{1}/FVC and FVC (McKay and Horvath 1994).

In reporting results to the study participant, a slightly more conservative (but more widely accepted and standardized) clinical interpretation will be utilized (Pellegrino et al. 2005), to minimize the false positive rate and the chance of unduly alarming subjects about possible obstructive or restrictive lung disease when it does not exist.
Figure 1. Universal flow diagram for interpretation of spirometry (adapted by R. McKay from McKay and Horvath 1994).

Biospecimen collection methods

Universal precautions will be observed through all biospecimen collection, to minimize the possibility of infectious disease communicability. These precautions will consist of use of nitrile gloves that are discarded after each study participant is processed, lab coats, the frequent washing of hands, and the use of medical waste and sharps disposal containers.

All biological specimens (blood, sputum, nasal and buccal cell swabs) will be collected at the middle or end of the shift for which the PBZ exposure measurements were collected. Two specimen sample types will be collected: one for analyses to be conducted within a matter of
days (e.g., CBC with differential) and one for storage to conduct later analyses (e.g., cytokines and oxidative stress biomarkers, as well as cytogenetic markers). Biospecimens to be analyzed immediately will be sent via overnight mail to contract laboratories for analysis. Samples to be stored for future analyses will be prepared in the field as described in section “Sample Collection and Preparation”, and will be stored in a -20°C freezer in the FERV. These specimens will then be sent to NIOSH-HELD and will be stored in an ultra-low temperature (-80°C) freezer, according to National Cancer Institute’s “Best Practices for Biospecimen Resources” (http://biospecimens.cancer.gov/bestpractices/to/bcpsrd.asp).

Whole blood will be collected from each volunteer study participant after the spirometry and sputum collection are completed. There will be a total of 44 mL collected from each volunteer. This represents no more than about 1% of blood volume. Each study participant undergoing phlebotomy will be offered water or juice and a snack (e.g., crackers) after the blood withdrawal.

Induced sputum will be collected for each consenting subject who does not have contraindications or active respiratory infection, using the procedure outlined in an induced sputum evaluation among popcorn manufacturing workers (Akpinar-Elci et al. 2005), modified to use isotonic saline at lower nebulizer output rate, which has been shown to reduce the occurrence of side effects while producing sputum of acceptable quality (Loh et al. 2004). In brief summary, participants will first be screened to determine whether they meet the eligibility criteria for induced sputum. Contraindications for sputum induction include beta-blocker use, cardiac arrhythmia or angina, recent (within past three months) surgery or pneumothorax, pregnancy, or baseline FEV$_1$ <60% of predicted. Consenting participants who are eligible will
inhale for 12 minutes in total a sterile isotonic saline solution produced by a compressed-air generated nebulizer. Study participants will breathe in the mouthpiece and then, every two minutes, will be asked to remove the mouthpiece, spit saliva into a cup, take a deep breath through the mouthpiece and then cough the full breath along with sputum into a sputum cup. After six minutes of breathing through the nebulizer, the spirometry technician will measure the FEV\textsubscript{1} of the participant (once if within 80\% of baseline and twice if less than 80\% of baseline). If two consecutive measurements of FEV\textsubscript{1} indicate a 20\% drop (compared to baseline), then the sputum induction procedure will be halted and the participant administered a bronchodilator.

If sputum cannot be induced, or its induction is contraindicated, or the participant does not consent to sputum induction, the technician will collect buccal and nasal swipe samples of material from the inside of the study participant’s nose and cheek, respectively. Two separate swabs will be used for this procedure. A sterile swab will be used to brush the inside of one cheek or both nostrils for 10 seconds. The swab specimens will be dissolved in a cell-specific medium and processed for biomarker analyses.

Samples will be de-identified in the field: the worker will be assigned a dummy identifying number, based on his or her PBZ sample. This number will be used to identify biological specimens in all analyses. The key to identify the worker will be retained by the project’s Principal Investigators and will be protected from disclosure according to NIOSH’s standard procedures under the Privacy Act.
It is unlikely that any medical emergencies will result from the spirometry or medical examinations, or from the collection of blood, nasal, or buccal cells. Sputum induction with isotonic saline carries a very small risk of induction of bronchial spasm or reduction of FEV\textsubscript{1} [2.7% for hypertonic saline in a recent study of biomarkers of airways disease in a group of microwave popcorn manufacturing workers (Akpinar-Elci et al. 2005), and 0% in an evaluation among 16 subjects who underwent sputum induction with isotonic saline (Loh et al. 2004)]. A metered-dose inhaler of a beta-adrenergic receptor agonist (albuterol, e.g., Proventil) bronchodilator will be available to counteract any bronchospasm that occurs. A NIOSH physician who is board-certified in Internal Medicine and/or Occupational Medicine and has extensive experience in carrying out medical procedures in field studies will be present onsite during these procedures to administer the bronchodilator if necessary and assist in handling any adverse reactions. One of two NIOSH physicians (either Marie De Perio, MD, or Douglas Trout, MD, MHS) will accompany the study team on each site visit. Dr. De Perio is a licensed practicing physician board-certified in Internal Medicine and Infectious Diseases. Dr. Trout is also a licensed practicing physician and is Board-certified in Internal Medicine and Occupational Medicine. Drs. De Perio and Trout have each served as lead physician on many field investigations at NIOSH. If one of these physicians is not available to participate in a site visit, then a NIOSH physician with similar qualifications will be identified to join the team, and a request to add the physician will be made to the NIOSH Institutional Review Board (IRB) via email. In addition, the project officers are trained by the American Red Cross in Community First Aid and Safety, including CPR. All members of the testing team will be provided with a copy of the emergency plan (Appendix IV).
Questionnaire administration

In order to determine if the study participant has any contraindications for spirometry or sputum induction, and to properly interpret the spirometry results as well as biomarkers of exposure and early effect, a questionnaire (see Appendix II) will be administered by NIOSH investigators to study participants after informed consent is obtained. The questionnaire will begin with the American Thoracic Society’s 1978 Adult Questionnaire (http://www.cdc.gov/niosh/atswww.txt) and will include or be supplemented with the following information: medical and smoking histories, exposure to passive (secondhand) smoke, exposures to agents that may cause adverse pulmonary effects (e.g., see Table 4), and history of diseases or conditions that may interfere with the interpretation of the biomarker results. For example, questions regarding dyspnea (resting or under exertion) or weight loss (Kaneko et al. 2010) are necessary for interpreting spirometry or certain biomarkers as possible early indicators of pulmonary fibrosis; other collagen diseases such as cystic fibrosis, scleroderma, hepatitis C, which might cause elevations in some of the fibrosis biomarkers. The NIOSH physician on the field study (Dr. Marie De Perio or Dr. Douglas Trout) will make the determination regarding the subject’s eligibility for spirometry and sputum induction, based on the questionnaire responses and the results of the medical examination, evaluated against the contraindications for spirometry.

EPIDEMIOLOGIC ANALYSIS

All statistical analyses will be conducted using the latest version of SAS (SAS, Inc, Cary, NC). Statistical analyses such as multiple linear regression models will relate different aspects of measured CNT and CNF PBZ exposure (e.g., respirable mass concentration, particle
concentration and surface area) to the outcome measures, which include spirometry measurements (e.g., FVC as percent of predicted), systolic and diastolic blood pressure, and potential biomarkers of early effect. These models will be adjusted for covariates such as age, gender, race, other workplace exposure, relevant exposure factors, and smoking history. Appropriate methods will be used to ensure the validity of the regression models employed (e.g., log-transformations of exposure or outcome data to ensure normality of the model residuals and good-fitting exposure-response associations). Because of the large number of biomarkers involved, consideration will be given to appropriate methods for adjusting for multiple comparisons.

For dermal exposure, the exposure-response analysis approach will be similar to that described above to evaluate associations between PBZ exposures and health outcomes and biomarkers, except that analyses will exclude the markers of early pulmonary fibrosis (e.g., KL-6) and measures of lung function.

For the leukocyte stimulation study, for each of the 46 evaluated biomarkers \((b)\), a stimulation effect for the \(i\)th individual \((\text{StimEff})_i\) will be calculated as follows:

\[
\text{StimEff}_b^i = \frac{([\text{Stimulated\_cells}]_b^i - [\text{Null\_cells}]_b^i)}{\text{Total\_leukocytes}_i}
\]

Where \([\text{Stimulated\_cells}]_b^i\) is the biomarker \(b\) concentration in the supernatant from stimulated cells for individual \(i\);

\([\text{Null\_cells}]_b^i\) is the biomarker \(b\) concentration in the supernatant from null (unstimulated) cells for individual \(i\);

\(\text{Total\_leukocytes}_i\), the total number of leukocytes for individual \(i\).
Normalization to total leukocyte count is used for each individual because the stimulation is based on a consistent volume (1 mL) of whole blood instead of leukocyte number, and exposure to CNT or CNF may result in an increase in total leukocyte count (e.g., Hulderman et al. 2012), which is not of interest in this study.

The StimEff variable calculated for each biomarker will be used as a dependent variable in a multiple linear regression model evaluating the association with each individual’s CNT or CNF exposure level (based on mass, particle concentration, or surface area), after adjusting for potential confounders such as age, gender, race, other workplace exposure, relevant exposure factors, and smoking history. Consideration will be given to grouping the biomarkers into classes, based on presumed mechanism or type of effect (e.g., inflammatory, oxidative stress, coagulation, immune), to determine whether similar biomarkers elicit similar response patterns.

**POWER ANALYSIS**

A power analysis was conducted for the FVC as a percent of predicted (as a measure of possible restrictive lung disease), and for biomarkers that have been evaluated in studies of other potentially hazardous exposures. In the design of the present study, multiple linear regression is proposed (after Cohen 1988) to evaluate the relation between CNT or CNF exposure and the spirometry or biomarker result, controlling for several potential confounders, including age, race/ethnicity, gender, smoking and possible exposure to sources of non-engineered nanomaterials or other potentially hazardous agents.

Power analysis calculations for FVC were based on simulations of effect sizes in SAS, followed by power calculations of the simulated effect size using Power Analysis and Sample Size Software (PASS 11; NCSS Statistical Software, Kaysville UT). For the biomarker analyses,
just PASS 11 software was used. For all sample size analyses, a Type I error rate ($\alpha$) of 0.05 and a power (1-$\beta$) of 0.8 were assumed. For simplicity, the exposure metric employed for FVC power analyses was background-corrected EC concentration ($\mu g/m^3$) as an eight-hour TWA, measured in at least one PBZ sample for the participant or his or her group representative. Data from the Phase II feasibility study (Dahm et al. 2012, supplemented with the related NIOSH research of Birch et al. 2011 and Evans et al. 2010, as well as more recent information) were used to estimate exposure distributions. The exposure distribution for the entire workforce group was assumed to be right-skewed, with 30% having an assigned exposure value of zero, 20% having exposure values between 0.5 and 2.0 $\mu g/m^3$ (mean 1.25 $\mu g/m^3$), 20% having exposure values between 2.0 and 4.0 $\mu g/m^3$ (mean 3.0 $\mu g/m^3$), 20% having exposure values between 4.0 and 8.0 $\mu g/m^3$ (mean 6.0 $\mu g/m^3$), and 10% having exposure values of greater than or equal to 8.0 $\mu g/m^3$ (assumed mean 12 $\mu g/m^3$).

For the spirometry simulation calculations, a reduction of FVC (as a percent of age-, race/ethnicity-, gender- and height-predicted) was assumed to be 1% for each increase of 1 $\mu g/m^3$ of background-corrected EC exposure. Unexposed (non-smoking) subjects were assumed to have FVCs averaging 100% of predicted, with a standard deviation of 10%. This standard deviation was also assumed to apply at the higher EC levels. It was assumed that smoking and exposure to other dusts or non-engineered nanoparticles would be adjusted-for as a confounder (and would together explain 30% of the variability in FVC), but it was also assumed that there would be no effect modification by these factors. In simulations using the exposure and effect scenario given above for the EC distribution in the studied group and its association with FVC decrement, a mean $R^2(T)$ of 0.12 is found (standard deviation=0.053) (Fig. 2).

For a multiple regression model controlling for two confounding variables (smoking and
exposure to non-CNT dusts or PM) that together result in a coefficient of multiple determination from “control variables” \([R^2(C)]\) of 0.30, a sample size of 41 would give a power of 0.80 to detect an increase of 0.12 in the coefficient of multiple determination [from “test variables”, \(R^2(T)\)] (Fig. 3). Although power to detect a 1% drop in FVC per 1 unit increase in EC might be adequate with 41 subjects, the estimate of \(R^2(T)\) of 0.12 is quite variable as shown above, with a standard deviation of nearly half the mean. A more conservative estimate of \(R^2(T)\) would be 0.05, which would have 80% power with 100 study subjects. Thus, with 100 study participants, power appears more than adequate to detect an FVC decrement of at least 1% per \(\mu\)g/m\(^3\) of background-corrected EC exposure.

For the biomarker analyses, power analyses were conducted assuming that six confounding variables (age, sex, race/ethnicity, body mass index, smoking and exposure to non-engineered nanomaterial or other agents) are to be controlled in the analyses for five biomarkers of primary interest as measures of early pulmonary fibrosis (KL-6), inflammation and cardiovascular disease or coagulation (IL-6, fibrinogen), and oxidative stress (GPx and 8-isoprostane), and that these six factors together have an \(R^2(C)\) of 0.40. The five biomarkers of interest were selected either because they are good indicators of pre-clinical pulmonary fibrosis (i.e., KL-6), or because they showed associations with CNT or other nanomaterial exposures in the only nano-epidemiology study reported publicly to date (Liou 2011). Under this scenario, a sample size of 100 would give a power of 0.80 to detect an increase of 0.07 in the coefficient of multiple determination [from “test variables”, \(R^2(T)\)] (Fig. 4). No published biomarker studies have been reported on CNT or CNF-exposed populations, so estimates of expected effect are difficult to make. However, more generally, required sample sizes have been found to be small for some biomarkers: Wones et al (1995) presented details on sample size estimation in
biomarker studies; they concluded that 12 participants per group were needed to show a twofold increase in hprt frequency at $\alpha=0.05$ with 80% power. Thus, the calculations performed above suggest that a sample size of 100 will be sufficient to observe a relatively small increase in predictive capability of background-corrected values for the biomarker outcome variables.

Figure 2. Simulated forced vital capacity (FVC), as percent predicted, resulting from a decrement of 1% per unit of background-corrected elemental carbon exposure, assuming a standard deviation of 10% in FVC among the unexposed.
Figure 3. Effect measure $[R2(T)]$ detectable for forced vital capacity (as a percent of age-, race-, gender- and height-predicted) related to background-corrected elemental carbon at a power of 0.8 and an alpha of 0.05.
Figure 4. Effect measure \([R^2(T)]\) detectable for a set of five biomarkers (adjusted for six confounding variables) related to background-corrected elemental carbon at a power of 0.8 and an alpha of 0.05.
HUMAN SUBJECTS PROTECTIONS

This protocol will be reviewed by the NIOSH IRB at project initiation and yearly thereafter. The NIOSH IRB review process provides mechanisms for reporting adverse events. For the cross-sectional study of early health effects and biomarkers, subjects will be informed of study objectives, procedures, the voluntary nature of their participation, and the risks and benefits of participating. Written informed consent (Appendix I) will be obtained and preserved for documentation. The informed consent document in Appendix I was reviewed for readability. It shows a Flesch Reading Ease score of 60.6 (target is 60-80), and a Flesch-Kincaid grade level of 9.5. This grade level should be sufficiently readable for the workers in this study, most of whom have bachelor-level or higher degrees.

A waiver of consent for the records obtained from companies in the study will be based on 8(c)(1) of the Occupational Safety and Health Act of NIOSH, which permits statutory authority to collect these records.

Confidentiality

All data collected for this study will be maintained in accordance with the Federal Privacy Act of 1974. All data for the study will be maintained in accordance with NIOSH and CDC policies on data security for sensitive but unclassified data, including restricted access to study team members with a “need to know” each data source. Hardcopy study records will be maintained in locked rooms or file cabinets with access restricted to study team members with a justified need to maintain access.
Worker notification

All study subjects participating in this cross-sectional study will be informed of their individual results of the exposure assessment, medical evaluation, and biomarker measurements. Study participants will be notified on the day of the medical exam about their height, weight, waist circumference, blood pressure, and heart rate (Addendum 1). They will be notified within one month of all clinically relevant medical findings (the above measures, plus BMI, CBC, and spirometry results; Addendum 2). They will be notified within approximately 6 months (depending on time required for TEM analyses) of their exposure assessment results (Addendum 3). Participants will be notified of the results of their biomarker analyses within 3 months of completion of these analyses (at the end of all data collection), and they will also be sent a summary of study findings. Notification letters for the biomarker analysis results and summary of study findings will be submitted to the NIOSH IRB for review.

Study Risks and Benefits

Assessment of Potential Benefits

Some of the information being collected during this study (i.e., blood pressure, CBC with differential, spirometry findings) is clinically relevant for the individual participant and is therefore of benefit to the individual. The participant and (if the participant consents) his or her personal physician will receive written results of these tests within one month of the site visit, along with appropriate interpretation of their clinical significance. The study participants will also receive a report on the results of his or her carbon nanotube or nanofiber exposure measurements, conducted via personal breathing zone and dermal sampling, together with an interpretation of their meaning with respect to available guidelines or standards.
More generally, this study will provide information on whether workplace exposure to carbon nanotubes and nanofibers is related to measures and biomarkers of early pulmonary and cardiovascular health effects. Individual participants will be notified of the results of the overall study. If positive associations are found, the participants’ health may benefit by early intervention to reduce the chances of progression to actual disease. Most of the biomarkers have not been clinically validated; however, some (blood pressure, pulmonary function measured by spirometry, complete blood count with differential) are clinically relevant. The study participants will receive individual notification of the results of all clinically relevant tests and results, with an indication of results that are outside of reference ranges or have clinical significance. The study participants will also receive information on their workplace exposure to CNT and CNF (and any other exposures that are measured), and of their individual results for any non-clinically relevant biomarkers together with reference ranges for unexposed study participants. Other workers (non-participants) who are exposed to CNT or CNF may also benefit from the findings of this study.

**Assessment of Potential Risks**

The risk to participants of this study is minimal. They include answering questions that may be sensitive (e.g., medications and history of illnesses). Respondents may choose not to answer any questions at any time without penalty. There is a slight risk of unintended disclosure of the data obtained from the questionnaire, medical examination and procedures, or biomarker analyses. This risk will be minimized as described below.

Spirometry and sputum induction may involve slight, temporary, discomfort. Some people feel lightheaded during or after performing spirometry or sputum induction, but this is
generally minor and goes away after sitting down. In some persons, spirometry or sputum induction is contraindicated because it can cause adverse health effects.

A small percentage (<3%) of people who have the induced sputum procedure with hypertonic saline may experience temporary bronchial spasm. The chances of this will be minimized by using isotonic saline, which has a much lower risk. Nevertheless, a NIOSH physician with extensive field medical experience and board certification in Internal Medicine and/or Occupational Medicine (either Marie De Perio, MD, or Douglas Trout, MD, MHS) will be monitoring the sputum induction procedure and will administer a bronchial dilator in the event that bronchospasm should happen to any study participants. During the blood draw, participants will feel a momentary prick when upon needle entry into the arm; some individuals feel lightheaded or dizzy when their blood is drawn. Infrequently, an individual faints. Swelling, bruising or discoloration may occur in the area where the needle was inserted; this will disappear in about a week.

Another disadvantage of all the clinical and biomarker tests is that a test result may be outside the range of "normal" even though nothing is wrong. Study participants may become worried or anxious about test results outside the normal range, even though such results may not indicate a clinical problem. To minimize this possibility, the letter communicating the findings of the individual biomarker analyses will emphasize the fact that the biomarker tests are useful for research only and have no clinical interpretation. The letter will use readily understandable examples (e.g., height) to describe the meaning of falling outside the normal range for a given measurement without a clinical (health) consequence.
Description of Measures Taken to Minimize Potential Risks

Risk of unintended disclosure of the data in the study will be minimized by utilizing only electronic records for the study (e.g., using a computer-assisted personal interview and “printing” questionnaires and spirometry results only to electronic portable document format files saved on password-protected, encrypted flash drives and laptops). Other steps being taken to minimize the risk of disclosure of personal data are described in the “Confidentiality” section on p. 62 above.

Extensive efforts are undertaken to reduce adverse effects of the medical procedures undertaken with study participants. They will be screened to minimize the chances of adverse health effects from spirometry, sputum induction, or phlebotomy, as described above. Standard procedures will be used to reduce infection and other adverse risks of phlebotomy. Risks of bronchospasm from sputum induction will be minimized by use of an isotonic saline solution with a low-flow-rate compressor nebulizer, rather than an ultrasonic (high-flow-rate) nebulizer with hypertonic solution (Loh et al. 2004). Risks will be further reduced by monitoring of FEV₁ via spirometry during the sputum collection process, to quickly identify any impairment of airway function.

All personally identifiable information for studies collected by NIOSH is accorded protections under the Federal Privacy Act. There is considerable physical security provided for individually identifiable information at NIOSH. This information is identified as “Sensitive but Unclassified”. Such data and records are stored in key-locked cabinets daily. Access to the information is on a “need-to-know” basis. The work area is in a building with 24-hour security guard service with access restricted to authorized keycard holders. The computer system is maintained through highly secured software. Users, authorized by the systems security officer, must enter user ID’s and passwords prior to achieving systems access. Furthermore, a security
access matrix is maintained within the branch that restricts access to study data to users on a need-to-know basis. Only NIOSH employees and their contractors who are directly working on this study will be permitted access to the data.

**Vulnerable populations**

The worksites included in this study involve adult working populations, and thus our study by design will exclude prisoners and children. Pregnant women are eligible to enroll and thus receive the benefits of the study; however, they will be excluded from the sputum induction procedure, as pregnancy is a contraindication. The blood collection volume is below the “minimal risk” threshold of 50 mL. Spirometry is not contraindicated during pregnancy. Pregnancy will be assessed by questionnaire.

**Risk versus Benefit Evaluation**

We will suggest a determination of the risks associated with this study to be minimal. As noted above, steps will be taken to minimize and address any distress or discomfort that participants may experience. Because the study participants will receive individual benefits from information obtained for the study, and the fact that results of this study may impact the health of the growing number of workers exposed to CNT and CNF, the anticipated benefits outweigh potential harm and discomfort to the study participants. Peer reviewers of the protocol were asked to specifically comment on whether the risks outweighed the benefits. The peer reviewers indicated that the benefits outweighed the risks. This study will be reviewed by the Human Subjects Review Board at NIOSH, which will be responsible for the determination of the level of risk, benefit, and whether benefits outweigh the risk of participating.
STUDY STRENGTHS AND LIMITATIONS

Limitations of the study include the following:

1) **The cross-sectional design precludes the establishment of temporality in exposure and effect.** For this reason, we are developing a protocol for a prospective cohort study, which will permit the evaluation of health effects from a population that is disease-free at the start of follow-up. However, given the short time period that most workers have been handling CNT and CNF, it is unlikely that elevated rates of disease (if they do occur) would be observable within the near future.

2) **The biomarkers of early effect to be used in this study have generally not been validated for clinical use.** Unfortunately, no clinically validated biomarkers of early effect have been developed for the outcomes of primary interest (pulmonary fibrosis, cardiovascular disease, and cancer). This limitation may reduce the interpretability of any associations that are observed between exposure measurements and the biomarkers of early effect, although the incorporation of clinically validated tests such as spirometry as an indication of restrictive lung disease does serve to reduce this limitation. To further minimize this limitation, we will continue to search the literature to update information about the biomarkers we have selected and any new biomarkers that may show strong correlations between occupational exposures and early health effects as the study proceeds. Any changes or additions to the biomarkers being evaluated will be submitted to the NIOSH IRB for review. Any analyses not proposed specifically in this protocol will be conducted only on deidentified specimens from participants who consent to such use.

Despite the limitations discussed above, this study has the following strengths:

1) **The availability of concurrent exposure and biomarker/outcome data** to evaluate early possible pulmonary, genetic and cardiovascular effects associated with CNT and CNF
exposure in workers.

2) **Wide representation across a large number of companies** manufacturing or using CNT or CNF in the U.S. and across a range of exposure levels, relative to those for which health effects have been observed.

3) **A staged approach is being taken** to permit the rapid evaluation of early health effects among CNT or CNF workers, followed by a more thorough (and prospective) evaluation of clinical health effects. This will maximize both the utility of the data (making use of the most current biomarkers of exposure and effect) and the informativeness and representativeness of the cohort study.
REFERENCES

Akpinar-Elci M, Stemple KJ, Enright PL, Fahy JV, Bledsoe TA, Kreiss K, Weissman DN. 


72


http://icon.rice.edu


Li N, Nel AE. Feasibility of biomarker studies for engineered nanoparticles: what can be learned from air pollution research. J Occup Environ Med 2011; 53:S74-S79.


APPENDICES: DOCUMENTS PERTAINING TO CROSS-SECTIONAL BIOMARKER STUDY

Appendix I: Fact sheet and informed consent documents for biomarker study participants

Appendix II: Medical history questionnaire to be administered to biomarker study participants

Appendix III: Exposure factors related to company and employee (to be completed by NIOSH investigator)

Appendix IV. Emergency plan while conducting medical examinations and collecting biospecimens
Appendix I. Fact sheet and informed consent documents

Fact sheet for participants in the Study of US Workers Exposed to Carbon Nanotubes and Nanofibers

National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention
Cincinnati, OH
October 2012

Project officer contact information:
Mary K. Schubauer-Berigan, PhD
National Institute for Occupational Safety and Health
4676 Columbia Pkwy, MS-R15
Cincinnati, OH 45226
Phone: 513-841-4251
Email: zcg3@cdc.gov

Or

Matthew M. Dahm, MPH
National Institute for Occupational Safety and Health
4676 Columbia Pkwy, MS-R14
Cincinnati, OH 45226
Phone: 513-458-7136
Email: mdahm@cdc.gov

Why is this study being done?

The National Institute for Occupational Safety and Health (NIOSH) does studies to see whether possibly harmful substances in the workplace are related to health of the workers using these materials. NIOSH is doing this study to measure your workplace exposure to carbon nanotubes (CNT) and carbon nanofibers (CNF) and to measure factors related to your health, to see if they are related to CNT or CNF exposure. We are doing this study to look at early markers for the following possible health effects in this study:

- Lung disease, such as pulmonary fibrosis
- Heart disease, such as high blood pressure and atherosclerosis
- DNA changes and inflammation that may lead to cancer

We do not think that exposure levels in workplaces are high enough, or have happened for a long enough time, to cause these diseases at this time. Instead, we are looking at biomarkers of early possible effects in body tissues (blood, phlegm, and cheek or nasal cells). In this study, we will be comparing workers who have higher exposures to workers from the same workplaces who have lower or no exposures to CNT or CNF. We will be including 100 workers from at least ten worksites where CNT or CNF are manufactured and/or used, including yours.
What will I be asked to do in this study?

All parts of this study will be done during your normal workday, and you will not need to take time off from work to participate. We will visit your worksite for several days during one week. On this visit, we will measure your workplace exposure to CNT and/or CNF. This will involve your wearing a vest that has three small pumps that will collect samples in your breathing space. Your exposure will be measured in this way through your entire work shift for one or two days. We will also look at whether CNT or CNF may be present on your skin after your work shift by placing and then removing a small piece of adhesive tape on the skin of your fingers, palms and wrists. Back in the lab, we will look at the adhesive under a microscope to see if CNT or CNF are present on the sample.

One time during the study, you will also be asked to have an interview and a medical examination, which will take about 90 minutes. The interview will take between 15 and 30 minutes to complete. More information about the interview is given below. You will then be examined by a NIOSH physician, who will take your pulse and blood pressure, height, weight, and waist circumference, and will make sure you are healthy enough to take part in the rest of the study. You will be fully clothed for this examination, which will take 10-15 minutes.

After the medical exam, you will then have a lung function test, called spirometry, which will take about 15 minutes. In the lung function test, you will be asked to take a very deep breath and then blow it out as fast as you can, until all the air is gone (at least 6 seconds). The test will be repeated at least three times, to get an accurate enough result.

After the lung function test, you will be asked to provide a blood sample, totaling about 44 milliliters (3 tablespoons). A trained phlebotomist will draw a blood sample from the inside of your arm. This will take about 10 minutes.

Lastly, we will ask you to cough up a sputum (phlegm) sample, after first breathing in a fine mist of salt solution. This is a method that is often used in patients with asthma or emphysema to break up phlegm in the lungs. This will take about 30 minutes. A technician will recheck your lung function during this test.

What type of questions will I be asked in the interview?

You will be asked questions about
- Your current and past work and hobbies that have involved carbon nanotubes or nanofibers, chemicals, dusts or fumes
- Illnesses, especially those that affect your lungs or breathing
- The medicines you take (such as, aspirin, beta blockers and other drugs to lower blood pressure)
- Tobacco smoking use
- Alcohol use
You can choose not to answer any question, but if you do choose to answer, it is very important that you answer questions honestly.

What should I bring with me to the study?

- The name and address of your current doctor, if you would like us to send him or her a copy of your test results
- A list of the medicines you take (including non-prescription medicines, such as aspirin) or your medicine bottles

Can I eat or drink before my blood tests?

- Yes, you may eat and drink before your blood tests but you should not eat or drink the hour before giving a sputum sample.

Who should not participate?

- People with the following conditions should not have the spirometry test or have sputum induced with a nebulizer
  - Eye surgery in the last 3 months
  - Chest or abdominal surgery in last 3 months
  - Self or household member with tuberculosis exposure in the past year
  - History of aneurysm, collapsed lung, or detached retina
  - Heart attack or stroke within the past three months
- People with the following conditions should not have sputum induced
  - Current beta-blocker medication use
  - Certain cardiac conditions
  - Recent surgery or collapsed lung
  - Current pregnancy

If you have any questions about whether you have an illness or take medicine like this, you may discuss this with us when we visit for the survey.

What tests will be done on the biological specimens I provide?

We will do four types of tests:

- Tests to look for carbon nanotubes or nanofibers in your sputum or nasal samples
- Tests of possible DNA changes in your sputum, cheek, or nasal cells
- A complete blood count will be done to see if the number of red blood cells and different types of white blood cells is normal, and whether it is related to your carbon nanotube or nanofiber exposure.
- Tests to look for proteins that may be present at higher or lower levels than normal in your blood or sputum samples. Some of these proteins are possible early markers of lung or cardiovascular disease, but most of them do not have a clear medical meaning that tells useful information about your health, but they are useful for research purposes. We are
analyzing them to see if they show a possible relationship with exposure to carbon nanotubes and nanofibers in this research study.

- Research tests on your blood sample to see if your white blood cells respond to a stimulant in a way that is related to your carbon nanotube or nanofiber exposure.

Your blood and sputum samples will be split into different components for the various measurements. Since there will be more than enough of some components than is needed to run the tests, we would like your permission to store the extra for possible future research. This research might involve laboratory tests that have not yet been developed.

**Will my results be confidential?**

- Information collected about you will be protected by the Privacy Act of 1974. NIOSH has very strict practices in place to ensure that information we collect about you will be protected from unintentional disclosure. Under the Privacy Act, your information may be disclosed only to private contractors assisting NIOSH; to collaborating researchers under certain limited circumstances to conduct further investigations; to the Department of Justice in the event of litigation; and to a congressional office assisting individuals in obtaining their records. An accounting of the disclosures that have been made by NIOSH will be made available to you upon request. Except for these and other permissible disclosures expressly authorized by the Federal Privacy Act, no other disclosure may be made without your written consent.

- Your employer will not have access to any information you provide.

- The results of the study will be presented in a way so that you cannot be identified.

- With your permission, we will send some of your results (the ones that have clinical meaning, such as spirometry results and blood cell type profiles) to your doctor.

**Will I receive the results of the study?**

- You will receive a copy of your own test results.
  - Some of the tests have clinical (medical) meaning, in that they give an indication of your possible health status. These include your blood pressure, heart rate, complete blood count, and spirometry. We will inform you of the results of these tests in writing within one month of our visit. We will also send these results to your personal physician if you choose to have us do so.
  - Some of the tests do not tell us direct information about your health. These include the biomarker tests that we will be doing. We will send the results of these tests to you within six months after we finish measuring these tests. For these tests, we will tell you how your results compared to other people in the study with similar exposure as you, and to people with no exposure in the study.

- We will also let you know the overall results of the study.
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

You have been asked to take part in a CDC/NIOSH research study. We explain here the nature of your participation, describe your rights, and tell you how NIOSH will treat your records.

I. DESCRIPTION

1. Study Title: Industrywide Exposure Assessment and Epidemiologic Studies of Workers at Facilities Manufacturing, Distributing, or Using Carbon Nanotubes or Carbon Nanofibers in the United States

2. Sponsor and Project Officer: This project is to be done by the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention, 4676 Columbia Parkway, Cincinnati, OH 45226. NIOSH project officers are Mary Schubauer-Berigan, PhD and Matthew Dahm, MPH

3. Purpose and Benefits: The overall purpose of this study is to find out whether U.S. workers exposed to carbon nanotubes (CNT) or carbon nanofibers (CNF) have early signs of health effects, such as lung disease, heart disease, or cancer. At this time, no certain tests exist to tell whether any health effects are occurring in workers exposed to CNT or CNF. But this study will measure markers of early health effects to see if they may be related to CNT or CNF exposure.

The purposes of this study are:

(a) Measure Exposure: to measure exposure to CNT and CNF in the work place and in the personal breathing zone of the worker. We will also measure whether there are CNT or CNF particles on the worker’s skin after a work shift.

(b) Measure possible clinical health effects: to measure lung function using spirometry, and to measure blood pressure, heart rate, body mass index, waist circumference, and a complete blood count. We will interview you about your lung and heart health and past work. We will look at these health measures and interview responses together with the exposure measurements to see if CNT or CNF exposure is related to health status.

(c) Study Biomarkers: to look at proteins in some body tissues [sputum (phlegm), nasal cells or cheek swabs] and blood that may be biomarkers (early indicators) of health
effects. These could include genetic changes, lung impairment, or oxidative stress and inflammation. These biomarkers may be related to early lung disease, heart disease or cancer. We will look at these biomarker results to see if they are related to your measured CNT or CNF exposure.

Answers to these questions will help NIOSH, companies, and workers understand whether exposure to CNT or CNF may be related to early health effects in U.S. workers. Similar studies are being done in workers exposed to CNT or CNF in other countries.

The benefits to you from being in the study include:
(a) You will receive the results of your lung function test, blood pressure and heart rate tests, complete blood count tests, and other tests using blood, sputum, cheek cells or nasal swipes, and of your exposure measurements to CNT and/or CNF.

You will receive a verbal summary of the results of the lung function, blood pressure and heart rate tests on the day these tests are done. NIOSH will send you written results and copies of these tests and of the complete blood count within one month of your exam. We will also send suggestions (if any) for follow-up care to be discussed with your personal physician.

NIOSH will send you results of your personal air samples (or those of a co-worker performing the same job as you) within about 6 months of our site visit. NIOSH will send you all remaining test results done on your blood, sputum, nasal or cheek cells, within one month of us getting the results. It may take up to 18 months before we can share these results with you, because we must store these samples until all the workers are enrolled in our study. Study enrollment could take more than one year.

NIOSH will also send you the results of some research tests done on your samples, but the health importance of these tests is not known.

(b) Your being in the study may also help fellow workers and others exposed to CNT or CNF, as a result of what is learned from this study. NIOSH will provide you and your personal doctor (if you wish) with all findings. We will do this when the study is finished, or sooner, for some findings. You will also receive a copy of the study report along with a brief summary of the study results.

Besides the information about your exposure, health status, biomarker results, and the results of the study, which we will send to you, there are no other benefits to you of being the study.

II. CONDITIONS OF THE STUDY

1. All parts of this study will be done during your normal workday. You will not need to take time off from work to be in the study. Being in the study will involve:
i. Exposure measurements will be done at your workplace for two to three days. During this period, most of you will be asked to wear three small sampling pumps placed in the pockets of a fishing vest. The sampling pumps pull air through (1) a filter to measure amounts of elemental carbon (a marker for CNT or CNF) and (2) a filter used to count the number of structures containing CNT and CNF in the air you are breathing. We ask you to wear the pumps so we can measure the amount of CNT or CNF in the air around you. Other than the inconvenience, there should be no discomfort from wearing the pumps. We will also look at whether CNT or CNF may be present on your skin after your work shift by placing and then removing a small piece of adhesive tape on the skin of your fingers, palms and wrists. Back in the lab, we will look at the adhesive under a microscope to see if CNT or CNF are present on the sample.

ii. Once during the study, you will be asked to have an interview and medical examination, which will take about 90 minutes. The interview will take 30 minutes or less. The interview will be done in a private area near the NIOSH mobile study trailer or other private place. We will ask about your work history, medical history, lung and heart health, smoking, drinking, and other topics. We realize that some questions are sensitive. We need to ask them so we make sure that you are healthy enough to have the medical tests done. We also need them to interpret the results of your laboratory tests. Your answers to these and any other questions will remain private, as detailed in the Federal Privacy Act of 1974. You may decline to answer any question for any reason.

iii. After completing the interview, you will undergo a clinical examination by a physician. This examination will take about 10 minutes. This will take place in a private area of the NIOSH mobile study trailer. First, a physician will take a pulse and blood pressure reading. Your height, weight and waist circumference will be measured. The health-care staff will also ask some questions about your medical history. You will remain clothed during the examination, except that your height and weight will be measured in stocking-feet.

iv. After the clinical exam, you will have a spirometry test to measure lung function. This test will take about 15 minutes. This test measures the volume of air that you can blow out after taking a very deep breath. It also measures how fast you can blow out the air. The test will be repeated at least three times (but no more than eight times), in order to measure your lung function accurately. If you are feeling dizzy or faint at all while taking the test, you should stop and tell the spirometry technician.

v. After having the lung function test, you will be asked to provide a blood sample of 44 milliliters, about 3 tablespoons. The sample will be drawn from your inside arm, opposite the elbow, by a trained phlebotomist. This will take about 15 minutes.
Measurements that will be done on your blood samples include: (1) tests to measure any changes to the chromosomes within your white blood cells; (2) levels of certain proteins in your blood, which may be early indicators of lung or heart disease; (3) complete blood count; and (4) levels of some proteins produced in your blood indicating a possible inflammatory response to exposure.

vi. After collecting your blood sample, the technician will collect a sample of your sputum (phlegm), nasal or cheek cell. If you have a cold or asthma, you will not be able to give a sputum sample. If you are healthy enough for the sputum sample collection, you will breathe an aerosol of sterile saline solution through a mouthpiece. This will take about 30 minutes in total. Every two minutes, you will remove the mouthpiece, spit out saliva into a cup, take a deep breath through the mouthpiece, and then cough phlegm into a collection cup. You will do this five or six times. After the first six minutes, we will check your lung function again using spirometry.

If you cannot or choose not to give a sputum sample, we will ask to collect a swab of the inside of your cheek or nose.

We will do three tests on these sputum, nasal or cheek cell samples. First, we will measure whether any CNT or CNF structures can be observed in them. Second, we will measure any changes to the chromosomes within these cells. Third, we will measure some of the same proteins as in your blood samples.

vii. We will send you the results of each test after the analysis is completed. Some of the tests will be done after all the workers are enrolled in the study, so results may not be available for some time to come (a year or more). It may not be possible to do every test on every person in the study. Some of the tests are research tests and the significance of the results of these tests for indicating your health is not known. You will receive your test results for every test done on your blood, sputum, nasal or cheek samples, as well as information on the reference or group range found in the study. Reference ranges will be given for laboratory tests that have a known relationship to your health, and group ranges will be provided for tests that do not. If any of your test results fall outside the reference range, the findings will be reviewed by a doctor, who will explain the possible significance of the test result to you by letter or telephone. No tests for drugs or alcohol will be conducted on your blood, sputum, nasal or cheek samples.

Your blood, sputum, nasal and cheek samples will be split into different parts for the various measurements. Since there will be more than enough of some parts than is needed to run the tests, we would like your permission to use the extra for methods development or future research. These samples would be coded so that they cannot be linked back to your name. You will not receive any results of tests done on the samples. Any information that can be linked to the samples will be saved in broad categories (such as age 40-60) so that it cannot be used to identify you. If tests are developed using your samples that are clearly important to your
health, we will notify all participants about this and offer you the chance to have the test done and the individual results reported to you. When you sign the consent form to be in the study, you will be asked for separate permission to store your samples for future research.

2. There is a slight risk of unintentional disclosure of your information in the questionnaire and medical examination, but we will take extensive steps to minimize this risk. Your questionnaire data will be kept only in electronic form on a password-protected, encrypted laptop or thumb drive while away from NIOSH. Your data will be stored in highly protected electronic systems at NIOSH, and will be accessible only to those with a need to use the data for the purposes of the study.

There should be no discomfort from the blood pressure or heart rate readings, or the collection of nasal or cheek samples. There should be no more than slight discomfort from the lung function (spirometry) and induced sputum tests. Each spirometry test involves taking a deep breath and blowing all the inhaled air out as fast as possible. Some people feel lightheaded during or after the test. This is generally minor and goes away after sitting down. A small percentage (<3%) of people who have the induced sputum procedure may have temporary bronchial spasm. Bronchial spasm gives a sensation of tightness in the chest, difficulty breathing air out, or wheezing. Sometimes the only symptom is a drop in how well you can breathe out air quickly, measured by spirometry. We are minimizing the chance of this happening to you by using a less irritating form of saline solution. We will check your lung function with a spirometer midway through the sputum procedure to see if you may be having bronchial spasm. We will have a physician in the NIOSH mobile trailer to administer a bronchial dilator if this should happen to you. The bronchial dilator we will use is an emergency inhaler of albuterol (Proventil), which is often used by people with asthma. The other alternatives to using albuterol are (1) using a different type of beta-adrenergic receptor agonist that has a similar mode of action, or (2) doing nothing to reverse the bronchospasm. Doing nothing is not recommended as it may lead to further difficulties in breathing.

During the blood draw, you will feel a slight prick when the needle enters your arm; some people feel lightheaded or dizzy when their blood is drawn. Infrequently, an individual faints. You may also have swelling, bruising or discoloration in the area where the needle was inserted; this will disappear in about a week. Another disadvantage of all the clinical and biomarker tests is that a test result may be outside the range of "normal" even though nothing is wrong. This could result in a recommendation for further evaluation that, ultimately, may not have been necessary. If you have any reaction to or concerns about these procedures you should contact Mary Schubauer-Berigan, Ph.D., at (513) 841-4251.

3. There are no alternative procedures that would provide more information about your specific workplace exposures and possible associated health effects.

4. Injury or harm from this project is unlikely. But if it results, medical care is not provided, other than emergency treatment as described in item #2 above. If you are injured through negligence of a NIOSH employee you may be able to obtain compensation under Federal
Law. If you want to file a claim against the Federal government your contact point is: General Law Division of the Office of General Counsel, request the Claims Office: (202) 233-0233. If an injury or harm should occur to you as the result of your participation, you also should contact:

Mary Schubauer-Berigan, Ph.D.
Epidemiologist
NIOSH
Division of Surveillance, Hazard Evaluations, and Field Studies
4676 Columbia Parkway R15
Cincinnati, OH 45226
(513) 841-4251

or

Mark A. Toraason, PhD
Chair
NIOSH Human Subjects Review Board
4676 Columbia Parkway
Cincinnati, OH 45226
(513) 533-8591

5. If you have questions about this research, contact Dr. Mary Schubauer-Berigan at the address and phone number above. If you have questions about your rights as a member of this study, contact Dr. Mark A. Toraason, Chair, Human Subjects Review Board, at 513-533-8591.

6. Your participation in this study is voluntary and you may withdraw your consent and your participation in this study at any time without penalty or loss of benefits to which you are otherwise entitled. By agreeing to be in the study, you should understand that the most important parts of this study are the interview, the spirometry and blood pressure tests, the blood tests, and the exposure measurements.

7. NIOSH will provide you and your doctor (if you wish) with your results in writing when the study is completed, or sooner, if they have clinical meaning. NIOSH will send you a copy of the final report, and a brief summary of the study results. This summary report will not contain any information that could identify people in the study.

III. USE OF INFORMATION

This study is being done by The National Institute for Occupational Safety and Health (NIOSH). NIOSH is part of the Centers for Disease Control and Prevention (CDC), a government agency in the Department of Health and Human Services. We collect this information in order to learn about various kinds of work hazards that may influence the health of the American worker.
NIOSH is allowed to collect and keep information about you, including your results from this study along with your social security number, because of two laws passed by Congress. These laws are:

1. The Public Service Act, Section 301 (42 U.S.C. 241);

The information you supply is voluntary and there is no penalty for not providing it. You are free to choose not to be in this study. It is up to you. We collect your SSN so that we can link your information with your work history records, and so that we can find your updated mailing address so that we can notify you of your individual results and about overall study findings.

The data from this study will be used to evaluate associations between early lung and heart disease and other health effects and exposure to carbon nanotubes and carbon nanofibers. Data will become part of CDC Privacy Act system 09-20-0147, "Occupational Health Epidemiological Studies" and may be disclosed to private contractors assisting NIOSH; to collaborating researchers under certain limited circumstances to conduct further investigations; to the Department of Justice in the event of litigation; and to a congressional office assisting individuals in obtaining their records. An accounting of the disclosures that have been made by NIOSH will be made available to you upon request. Except for these and other permissible disclosures expressly authorized by the Federal Privacy Act, no other disclosure may be made without your written consent.
IV. SIGNATURES

I have read this consent form and I agree to participate in this study (Title: Industrywide Exposure Assessment and Epidemiologic Studies of Workers at Facilities Manufacturing, Distributing, or Using Carbon Nanotubes or Carbon Nanofibers in the United States)

PARTICIPANT____________________________________DATE_______
(signature)

I give permission for extra amounts of my blood, sputum, nasal or cheek specimens to be stored and used for future research in laboratory tests. I understand that my name and identification number will be taken off these stored specimens and that I will not receive the results of any of the research tests. I understand I will not be contacted again before these analyses are performed.

_____ GIVE PERMISSION   _____ DO NOT GIVE PERMISSION

PARTICIPANT____________________________________DATE_______
(signature)

PARTICIPANT’S
NAME:____________________________________________________
Address:_________________________________________________
City:________________________State:_______Zip:____________
Phone:(____)________________
Email address:________________________
Participant Social Security number:__________________
Participant ID number:________

I, the NIOSH/CDC representative, have accurately described this study to the participant.

REPRESENTATIVE_________________________________DATE_______
(signature)
REQUEST AND AUTHORIZATION FOR RELEASE OF INFORMATION

I, __________________________________________, request and permit NIOSH/CDC to inform the doctors or health care facilities whose names and addresses I have entered below of any significant findings from this study that concern me. (Do not leave blank. Write "NO" where you do not wish to give a name and address).

1. My personal doctor(s):

Dr. ________________________________

Street ________________________________

City ________________________________ State ___ Zip

2. Other doctors or health care providers:

Dr. ________________________________

Street ________________________________

City ________________________________ State ___ Zip
Appendix II. Questionnaire for participants in study of lung and cardiovascular function, and biomarkers of early health effects among CNT and CNF workers
(necessary for interpretation of biomarker results)

This questionnaire is based almost entirely on previously validated and tested questionnaires. Questions pertaining to respiratory symptoms are obtained from the American Thoracic Society’s 1978 Adult Questionnaire. Questions related to fitness for undergoing spirometry are drawn from the National Health and Nutrition Examination Survey Respiratory Health Spirometry Procedures Manual (NHANES 2008). All other questionnaire sections are from the NIOSH Immunotox Questionnaire (somewhat abridged). Questions on diseases with known renal effects (diabetes, gout, etc.) have been added to the disease sections in the same format as the questions already in those sections. Questions on lifetime alcohol consumption, condensed from the NIOSH Upper Midwest Health Study, have been added to the alcohol section (the Immunotox Questionnaire asks only about alcohol consumption in the past six months).

INTERVIEWER: PLEASE READ THIS TO PARTICIPANT PRIOR TO BEGINNING INTERVIEW

If you are concerned about providing sensitive information or have questions about participating, feel free to discuss this with me now. If you choose not to participate, you can quit now or at any time during the study. You may decline to answer any question, for any reason, and continue with the rest of the interview.

The purpose of this questionnaire is to obtain information about factors other than carbon nanotube or nanofiber exposures at your present job that might affect your health. The subjects that will be covered are your personal characteristics (that is, your birth date, race or ethnicity, and sex), your work history involving exposure to carbon nanotubes or nanofibers or other chemicals, dusts or fumes at (name of company under study), your hobbies, your history of certain illnesses, your use of certain prescription medications, your tobacco smoking habits, and your alcohol consumption patterns.

The information you provide will be protected under the Federal Privacy Act. Your personal information will not be shared with anyone in a way that allows you to be identified. We ask about your Social Security Number (SSN) so that we can link information about your health to your work history records at your company. However, you do not have to provide us your SSN.

1. STUDY ID NUMBER: _____ _____ _____ _____ (completed by NIOSH)

2. NAME: a. ___________________________ b. ____ c. ___________________________
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

3. ADDRESS: a. ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________ __________
d. |__| Asian (0. No; 1. Yes; 8. Refused; 9. Don’t know)
e. |__| Native Hawaiian or other Pacific Islander (0. No; 1. Yes; 8. Refused; 9. Don’t know)

11. **WOULD YOU DESCRIBE YOURSELF AS OF HISPANIC OR LATINO/LATINA ORIGIN?**
   |__| (0. No; 1. Yes; 8. Refused; 9. Don’t know)

12. Current height? a. |__| feet  b. |__|__| inches

13. Current weight? |__|__|__| pounds  (888=refused to answer, 999=don’t know)

14. What is your level of schooling? |__| (0 = None 1 = 1-7 years, 2 = elementary school graduate, 3=9-11 years, 4= high school graduate, 5= vocational school, 6=some college, 7=college graduate, 8=postgraduate, 9=refused)

**NOW I WOULD LIKE TO ASK YOU ABOUT SYMPTOMS THAT PERTAIN MOSTLY TO YOUR CHEST. PLEASE ANSWER YES OR NO IF POSSIBLE. IF A QUESTION DOES NOT APPEAR TO BE APPLICABLE TO YOU, LET ME KNOW. (For questions 15-48, if the participant is in doubt about whether his or her answer is yes or no, record no.)**

**15. COUGH**

a. Do you usually have a cough?  (Count a cough with first smoke or on first going out-of-doors. Exclude clearing of throat.) [If no, skip to question 15c.]  
   |__| 0. No; 1. Yes; 8. Ref

b. Do you usually cough as much as 4 to 6 times a day, 4 or more days out of the week?  
   |__| 0. No; 1. Yes; 8. Ref

c. Do you usually cough at all on getting up, or first thing in the morning?  
   |__| 0. No; 1. Yes; 8. Ref

d. Do you usually cough at all during the rest of the day or at night?  
   |__| 0. No; 1. Yes; 8. Ref

**IF YES TO ANY OF THE ABOVE (15a-d), ANSWER THE FOLLOWING:**
**IF NO TO ALL, SKIP TO 16a.**
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

\[\begin{align*}
\text{e. Do you usually cough like this on most days for 3 consecutive months or more during the year?} & \quad \boxed{[0; 1; 8]} \\
\text{f. For how many years have you had this cough?} & \quad \boxed{[\text{Number of years}]} \\
\end{align*}\]

16. PHLEGM

\[\begin{align*}
\text{a. Do you usually bring up phlegm from your chest?} & \quad \boxed{[0; 1; 8]} \\
& \quad \text{(Count phlegm with the first smoke or on first going out-of-doors. Exclude phlegm from the nose. Count swallowed phlegm)} \\
& \quad \text{[If no, skip to 16c.]}
\end{align*}\]

\[\begin{align*}
\text{b. Do you usually bring up phlegm like this as much as twice a day, 4 or more days out of the week?} & \quad \boxed{[0; 1; 8]} \\
\text{c. Do you usually bring up phlegm at all on getting up or first thing in the morning?} & \quad \boxed{[0; 1; 8]} \\
\text{d. Do you usually bring up phlegm at all during the rest of the day or at night?} & \quad \boxed{[0; 1; 8]} \\
\end{align*}\]

\[\text{IF YES TO ANY OF THE ABOVE (16a-d), ANSWER THE FOLLOWING: IF NO TO ALL, SKIP TO 17a.}\]

\[\begin{align*}
\text{e. Do you bring up phlegm like this on most days for 3 consecutive months or more during the year?} & \quad \boxed{[0; 1; 8]} \\
\text{f. For how many years have you had trouble with phlegm?} & \quad \boxed{[\text{Number of years}]} \\
\end{align*}\]

17 EPISODES OF COUGH AND PHLEGM

\[\begin{align*}
\text{a. Have you had periods or episodes of (increased*) cough and phlegm lasting for 3 weeks or more each year?} & \quad \boxed{[0; 1; 8]} \\
& \quad \text{*(For individuals who usually have cough and/or phlegm)}
\end{align*}\]
IF YES TO 17a:

b. For how long have you had at least 1 such episode per year?  Number of years

==============================================================================

WHEEZING

18a. Does your chest ever sound wheezy or whistling:
   1. When you have a cold?  0. No; 1. Yes; 8. Ref
   2. Occasionally apart from colds?  0. No; 1. Yes; 8. Ref
   3. Most days or nights?  0. No; 1. Yes; 8. Ref

IF YES TO 1, 2, OR 3 IN 18a:

b. For how many years has this been present?  Number of years

19a. Have you ever had an ATTACK of wheezing that has made you feel short of breath?  0. No; 1. Yes; 8. Ref

IF YES TO 19a, ANSWER 19b-d (IF NO, SKIP TO 20):

b. How old were you when you had your first such attack?  Age in years (888 Ref; 999 Don’t know)

c. Have you had 2 or more such episodes?  0. No; 1. Yes; 8. Ref

d. Have you ever required medicine or treatment for the(se) attack(s)?  0. No; 1. Yes; 8. Ref

==============================================================================

BREATHELESSNESS
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

20a. Are you disabled from walking?  
   IF YES TO 20a, answer 20b (IF NO OR REFUSED, SKIP TO 21a)  
   b. Is this disability due to heart or lung disease?  
      IF NO to 20b, SKIP TO 22

21a. Do you have shortness of breath when hurrying on the level or walking up a slight hill?  
   IF YES TO 21a, ANSWER 21b-e (IF NO, SKIP TO 22):
   b. Do you have to walk slower on the level than people of your age because of breathlessness?  
      c. Do you ever have to stop for breath when walking at your own pace on the level?  
      d. Do you ever have to stop for breath after walking about 100 yards (or after a few minutes) on the level?  
      e. Are you too breathless to leave the house or breathless on dressing or undressing?

22. If you get a cold, does it usually go to your chest? (Usually means more than 1/2 the time)  
   IF YES TO 22, ANSWER 22b (IF NO, SKIP TO 23a):
   b. Do you produce phlegm with any of these chest illnesses?  
      c. In the last 3 years, how many such illnesses, with (increased) phlegm, did you have which

CHEST COLDs AND CHEST ILLNESSES

23a. During the past 3 years, have you had any chest illnesses that have kept you off work, indoors at home, or in bed?  
   IF YES TO 23a, ANSWER 23b-c (IF NO, SKIP TO 23d):
   b. Did you produce phlegm with any of these chest illnesses?  
      c. In the last 3 years, how many such illnesses, with (increased) phlegm, did you have which

105
lasted a week or more?

d. Do you currently have a cold or other upper airway infectious disease?   ___ 0. No; 1. Yes; 8. Ref

==============================================

PAST ILLNESSES

24a. Have you ever had pneumonia (include bronchopneumonia)?   ___ 0. No; 1. Yes; 8. Ref

   IF YES TO 24a, ANSWER b and c (IF NO, SKIP TO 25a):
   b. Was it confirmed by a doctor?   ___ 0. No; 1. Yes; 8. Ref

   c. At what age did you first have it?    ___ ___ ___ Age in years (888 Ref; 999 Don’t know)

25a. Have you ever had hay fever or respiratory allergies?   ___ 0. No; 1. Yes; 8. Ref

   IF YES TO 25a, ANSWER b and c (IF NO, SKIP TO 26a):
   b. Was it confirmed by a doctor?   ___ 0. No; 1. Yes; 8. Ref

   c. At what age did it start?    ___ ___ ___ Age in years (888 Ref; 999 Don’t know)

26a. Have you ever had chronic bronchitis?   ___ 0. No; 1. Yes; 8. Ref

   IF YES TO 26a, ANSWER b-d (IF NO, SKIP TO 27a):
   b. Do you still have it?   ___ 0. No; 1. Yes; 8. Ref

   c. Was it confirmed by a doctor?   ___ 0. No; 1. Yes; 8. Ref

   d. At what age did it start?    ___ ___ ___ Age in years (888 Ref; 999 Don’t know)

27a. Have you ever had emphysema?   ___ 0. No; 1. Yes; 8. Ref
IF YES TO 27a, ANSWER b-d (IF NO, SKIP TO 28a):

b. Do you still have it?  
   __| 0. No; 1. Yes; 8. Ref

c. Was it confirmed by a doctor?  
   __| 0. No; 1. Yes; 8. Ref

d. At what age did it start?  
   __|__|__ Age in years (888 Ref; 999 Don’t know)

28a. Have you ever had asthma?  
   __| 0. No; 1. Yes; 8. Ref

IF YES TO 28a, ANSWER 28b-e (IF NO, SKIP TO 29a):

b. Do you still have it?  
   __| 0. No; 1. Yes; 8. Ref

c. Was it confirmed by a doctor?  
   __| 0. No; 1. Yes; 8. Ref

d. At what age did it start?  
   __|__|__ Age in years (888 Ref; 999 Don’t know)

e. If you no longer have it, at what age did it stop?  
   __|__|__ Age in years (888 Ref; 999 Don’t know)

29. Have you ever had:

a. Any other chest illnesses?  
   If yes, please specify _____________________________  
   __| 0. No; 1. Yes; 8. Ref

b. Any chest operations?  
   If yes, please specify _____________________________  
   __| 0. No; 1. Yes; 8. Ref

c. Any chest injuries?  
   If yes, please specify _____________________________  
   __| 0. No; 1. Yes; 8. Ref

30a. Has a doctor ever told you that you had heart trouble?  
   __| 0. No; 1. Yes; 8. Ref
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

IF YES to 30a, ANSWER 30b-c (IF NO, GO TO 31a):

b. Have you had treatment for heart trouble in the past 10 years?
   |__| 0. No; 1. Yes; 8. Ref

c. Has a doctor ever told you that you have had a heart attack?
   |__| 0. No; 1. Yes; 8. Ref

31a. Has a doctor ever told you that you have high blood pressure?
   |__| 0. No; 1. Yes; 8. Ref

IF YES to 31a, ANSWER 31b (IF NO, GO TO 32a):

b. Have you had any treatment for high blood pressure (hypertension) in the past 10 years?
   |__| 0. No; 1. Yes; 8. Ref

NOW I WOULD LIKE TO ASK YOU ABOUT CERTAIN OTHER ILLNESSES OR HEALTH CONDITIONS YOU MAY HAVE HAD
(Note: affirmative responses to any questions 40-45, or stroke or heart attack within the past 3 months (questions 30c, 33a and 46), involve exclusions for spirometry):

<table>
<thead>
<tr>
<th>HAVE YOU EVER HAD: (Did you have any other?)</th>
<th>WHEN WAS THIS CONDITION FIRST DIAGNOSED?</th>
</tr>
</thead>
<tbody>
<tr>
<td>32a. Malignant tumor or cancer? (including leukemia or lymphoma)</td>
<td>b. Mo _____ c. Yr _______</td>
</tr>
<tr>
<td></td>
<td>(Describe):</td>
</tr>
<tr>
<td>33a. Diagnosed heart disease? (Describe):</td>
<td>b. Mo _____ c. Yr _______</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>HAVE YOU EVER HAD: (Did you have any other?)</td>
<td>WHEN WAS THIS CONDITION FIRST DIAGNOSED?</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>34a. <strong>Diabetes?</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>35a. <strong>Kidney disease? (Describe):</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>36a. <strong>Cystic fibrosis?</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>37a. <strong>Scleroderma?</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>38a. <strong>Lupus (systemic lupus erythematosus)?</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>39a. <strong>Any other autoimmune disease? (Describe):</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>40a. <strong>Eye surgery?</strong> (other than cosmetic surgery on the eyelid or skin around the eye)</td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>41a. <strong>Open chest or abdominal surgery?</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>42. <strong>Did you or anyone in your household have tuberculosis in the past year?</strong></td>
<td>b. Mo</td>
</tr>
<tr>
<td></td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>HAVE YOU EVER HAD:</td>
<td>WHEN WAS THIS CONDITION FIRST DIAGNOSED?</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>(Did you have any other?)</td>
<td></td>
</tr>
<tr>
<td>43. Has a doctor or other health professional told you that you had an aneurysm?</td>
<td></td>
</tr>
<tr>
<td>__</td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>44. Has a doctor or other health professional told you that you had a collapsed lung?</td>
<td></td>
</tr>
<tr>
<td>__</td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>45. Has a doctor or other health professional told you that you had a detached retina?</td>
<td></td>
</tr>
<tr>
<td>__</td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>46a. Has a doctor or other health professional told you that you had a stroke?</td>
<td>b. Mo __ __ c. Yr __ __ __ __</td>
</tr>
<tr>
<td>__</td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>47. In the past month, have you coughed up blood?</td>
<td></td>
</tr>
<tr>
<td>__</td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>48. Are you currently taking any prescription or nonprescription medication, including aspirin?</td>
<td></td>
</tr>
<tr>
<td>__</td>
<td>0. No; 1. Yes; 8. Ref</td>
</tr>
<tr>
<td>49. Please list all prescription and non-prescription medication you are currently taking.</td>
<td></td>
</tr>
<tr>
<td>50. Are you currently pregnant?</td>
<td></td>
</tr>
<tr>
<td>__</td>
<td>0. No; 1. Yes; 8. Ref 9. Don’t Know</td>
</tr>
</tbody>
</table>
NOW I WOULD LIKE TO ASK YOU ABOUT YOUR WORK HISTORY.

51. WHAT IS YOUR REGULAR WORK SHIFT? (If response is 4 or 8, skip to question 53)

52. WHAT ARE YOUR REGULAR SHIFT HOURS?
   From a. ____________ to b. ____________ (incl. am/pm)

53. HOW MANY HOURS PER WEEK DO YOU USUALLY WORK?   ___ Hours (888 – Refused; 999 – Don’t Know)

NOW I WOULD LIKE TO ASK YOU ABOUT THE JOBS YOU’VE HAD.

54. PLEASE DESCRIBE YOUR CURRENT JOB.

<table>
<thead>
<tr>
<th>a. WHAT IS THE NAME &amp; LOCATION (City/State) OF THE COMPANY?</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. WHAT IS YOUR DEPARTMENT?</td>
</tr>
<tr>
<td>c. WHAT IS YOUR JOB TITLE?</td>
</tr>
<tr>
<td>d. WHEN DID YOU START WORKING IN THIS JOB? (Month/Year)</td>
</tr>
<tr>
<td>e. WHAT ARE YOUR ACTIVITIES &amp; DUTIES?</td>
</tr>
<tr>
<td>f. DESCRIBE ANY CNT OR CNF, CHEMICALS, DUSTS, OR FUMES, INCLUDING DIESEL EXHAUST THAT YOU HAVE BEEN EXPOSED TO IN THIS JOB.</td>
</tr>
</tbody>
</table>

Start: ____________
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

55a. OTHER THAN YOUR CURRENT JOB, WHICH YOU JUST DESCRIBED, HAVE YOU WORKED IN ANY JOBS WHERE YOU WERE EXPOSED TO CARBON NANOTUBES (CNT) OR CARBON NANOFIBERS (CNF) OR CHEMICALS, DUSTS OR FUMES, INCLUDING DIESEL EXHAUST?

| 0 | No  (Go to #56)          |
| 1 | Yes (complete table below)|
| 8 | Refused (Go to #56)      |
| 9 | Don’t know (Go to #56)   |

PLEASE DESCRIBE THE OTHER JOB(S) YOU HAD WHERE YOU WERE EXPOSED TO CNT, CNF, OR CHEMICALS, DUSTS OR FUMES.

<table>
<thead>
<tr>
<th>b. WHAT WAS THE NAME &amp; LOCATION (City/State) OF THE COMPANY?</th>
<th>c. WHAT WAS YOUR DEPARTMENT?</th>
<th>d. WHAT WAS YOUR JOB TITLE?</th>
<th>WHEN DID YOU e. START AND f. STOP WORKING IN THIS JOB? (Month/Year)</th>
<th>g. WHAT WERE YOUR ACTIVITIES AND DUTIES?</th>
<th>h. DESCRIBE ANY CNT OR CNF, CHEMICALS, DUSTS, OR FUMES, INCLUDING DIESEL EXHAUST, THAT YOU WERE EXPOSED TO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start:</td>
<td>Start:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop:</td>
<td>Stop:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start:</td>
<td>Start:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop:</td>
<td>Stop:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iii.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
56a. **NOW I WOULD LIKE TO ASK YOU ABOUT YOUR HOBBIES.**

IN THE PAST 6 MONTHS, HAVE YOU HAD ANY HOBBIES WHERE YOU WORKED WITH ANY CHEMICALS OR HAD EXPOSURE TO CHEMICAL VAPORS, DUSTS OR FUMES, INCLUDING DIESEL EXHAUST? (SOME EXAMPLES MIGHT BE CARPENTRY OR FURNITURE REFINISHING, OR MAKING STAINED GLASS AS A HOBBY. INCLUDE ACTIVITIES ONLY IF YOU HAVE DONE THEM AT LEAST ONE HOUR A WEEK).

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>⬜ No   (Go to #56)</td>
<td>1</td>
<td>⬜ Yes   (Complete table below)</td>
<td>8</td>
<td>⬜ Refused   (Go to #56)</td>
<td>9</td>
</tr>
</tbody>
</table>

| b. WHAT IS THE EXACT NATURE OF THE HOBBY? | c. HOW MANY HOURS PER WEEK DID YOU DO THIS IN THE PAST 6 MONTHS? | d. WHAT CHEMICALS, DUSTS, OR FUMES (INCLUDING DIESEL EXHAUST) ARE YOU EXPOSED TO IN THIS HOBBY? |
IN THE NEXT PART OF THE QUESTIONNAIRE, I WOULD LIKE TO ASK YOU ABOUT YOUR TOBACCO HISTORY AND YOUR ALCOHOL CONSUMPTION PATTERNS.

<table>
<thead>
<tr>
<th>DID YOU EVER:</th>
<th>b. DO YOU CURRENTLY:</th>
<th>c. WHAT AMOUNT, ON AVERAGE, DO/DID YOU SMOKE/USE PER DAY? (one pack = 20 cigarettes)</th>
<th>d-e. DURING WHICH YEARS DID YOU:</th>
</tr>
</thead>
<tbody>
<tr>
<td>57a. SMOKE CIGARETTES (at least 100 in your lifetime)</td>
<td>□ 0. No; 8. Ref</td>
<td>□ 0. No; 1. Yes; 8. Ref</td>
<td>□□□□□□□□□□ cigarettes/day or □□ packs/day</td>
</tr>
<tr>
<td>58. SMOKE CIGARS (at least once/day for 6 months)</td>
<td>□ 0. No; 8. Ref</td>
<td>□ 0. No; 1. Yes; 8. Ref</td>
<td>□□□□□□□□□□ cigars/day</td>
</tr>
<tr>
<td>59. SMOKE A PIPE (at least once/day for 6 months)</td>
<td>□ 0. No; 8. Ref</td>
<td>□ 0. No; 1. Yes; 8. Ref</td>
<td>□□□□□□□□□□ pipesful/day</td>
</tr>
<tr>
<td>60. Chew tobacco or use snuff? (at least once/day for 6 months)</td>
<td>□ 0. No; 8. Ref</td>
<td>□ 0. No; 1. Yes; 8. Ref</td>
<td>□□□□□□□□□□ ounces /day</td>
</tr>
<tr>
<td>61a. LIVE with a regular smoker? (daily for 6 months or more)</td>
<td>□ 0. No; 8. Ref</td>
<td>□ 0. No; 1. Yes; 8. Ref</td>
<td>□□□□□□□□□□</td>
</tr>
</tbody>
</table>
62. Did you ever drink alcoholic beverages at least 12 or more times in a single year?
0 No (End interview) 1 Yes
8 Refused (End interview)

63. How many years total did you drink at least 12 or more alcoholic beverages in a single year?

___ Years, or 888 Refused, or 999 Don’t Know or year ___ to ___ or Age ___ to ___

64. During this period, about how many drinks (cans or glasses of beer, glasses of wine, shots of hard liquor straight or in a mixed drink) did you usually have per week? ___ (number of drinks, or 888 Refused or 999 Don’t know)

65a. OVER THE PAST 6 MONTHS, HAVE YOU CONSUMED, ON AVERAGE, AT LEAST 1 ALCOHOLIC BEVERAGE PER WEEK SUCH AS BEER, WINE, MIXED DRINKS, OR HARD LIQUOR?
0 No (End interview)
1 Yes (Continue) ->
2 Chooses not to respond (End interview)

b. PLEASE ESTIMATE HOW MANY TIMES, PER WEEK, YOU DRANK ALCOHOLIC BEVERAGES ON AVERAGE, OVER THE PAST 6 MONTHS.

___ per week

c. PLEASE ESTIMATE THE NUMBER OF DRINKS YOU HAVE, ON AVERAGE, ON EACH OCCASION.

___ (drinks; 888 Refused; 999 Don’t know)

Thank you! This completes the interview.
Appendix III: Exposure factors related to company and employee (to be completed by NIOSH investigator)

I. COMPANY-SPECIFIC INFORMATION

Company Name: ________________________________

Company Address (for facility under study): ________________________________

Site Visit Dates: ________________________________

Information related to use of carbon nanotubes (CNT) or carbon nanofibers (CNF)

Primary manufacturer of: ________ Secondary manufacturer of: ________________

CNT or CNF material #1:

Synthesis method, if a primary manufacturer: ________________________________

Precursor & catalyst used, if a primary manufacturer: ________________________________

CNT or CNF source, if a secondary manufacturer: ________________________________

Nominal aspect ratio: _____ Measured in bulk material

_____ Reported by company

Chemicals used in purification: ________________________________

CNT or CNF material #2:

Synthesis method, if a primary manufacturer: ________________________________

Precursor & catalyst used, if a primary manufacturer: ________________________________

CNT or CNF source, if a secondary manufacturer: ________________________________

Nominal aspect ratio: _____ Measured in bulk material

_____ Reported by company

Chemicals used in purification: ________________________________
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

CNT or CNF material #3:

Synthesis method, if a primary manufacturer: ________________________________

Precursor & catalyst used, if a primary manufacturer: ________________________________

CNT or CNF source, if a secondary manufacturer: ________________________________

Nominal aspect ratio: ______ Measured in bulk material

______ Reported by company

Chemicals used in purification: ________________________________

Other chemical or physical agents used at the facility: ________________________________

______________________________________________________________________________

Cleaning operations: ________________________________

Waste disposal practices: ________________________________

Personal protective equipment required: ________________________________

______________________________________________________________________________

Engineering and administrative exposure control devices and methods: ________________________________

______________________________________________________________________________

Other relevant information: ________________________________
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

II. WORKER-SPECIFIC INFORMATION (to be completed for every participant in the exposure assessment study)

NIOSH_ID: ________________________________

Length of shift (observed): ________________

Time spent per shift working directly with CNT or CNF (observed): __________________________

Time spent per shift potentially indirectly exposed to CNT or CNF (observed): ________________

Processes and tasks performed by employee:

Task #1: Description: ________________________________

Date: __________     Time: ________________

Monitored by NIOSH? (Yes/No) ______

If no, NIOSH_ID of employee performing similar task who was monitored: ________________

Form of CNT and CNF used (e.g., dry powder or liquid emulsion): _______________________

Personal protective equipment, used correctly?: ________________________________

Engineering controls used: ________________________________________________

Other potentially relevant information: ____________________________________________
Task #2: Description: 

Date: ____________  Time: ________________

Monitored by NIOSH? (Yes/No)  _____

If no, NIOSH_ID of employee performing similar task who was monitored: ________________

Form of CNT and CNF used (e.g., dry powder or liquid emulsion): ________________

Personal protective equipment, used correctly?: ________________

Engineering controls used: ________________

Other potentially relevant information: ________________

Task #3: Description: 

Date: ____________  Time: ________________

Monitored by NIOSH? (Yes/No)  _____

If no, NIOSH_ID of employee performing similar task who was monitored: ________________

Form of CNT and CNF used (e.g., dry powder or liquid emulsion): ________________

Personal protective equipment, used correctly?: ________________

Engineering controls used: ________________

Other potentially relevant information: ________________
Protocol for an Exposure Assessment and Epidemiological Study of U.S. CNT & CNF Workers

Task #4: Description: 

Date: ___________ Time: ______________

Monitored by NIOSH? (Yes/No)  _____

If no, NIOSH_ID of employee performing similar task who was monitored: ________________

Form of CNT and CNF used (e.g., dry powder or liquid emulsion): ____________________

Personal protective equipment, used correctly?: ________________________________

Engineering controls used: ________________________________

Other potentially relevant information: ________________________________

(use additional sheets as necessary)
Appendix IV. Emergency plan while conducting medical examinations and collecting biospecimens

AT FIELD LOCATIONS:

1. Call for help from another field team member.
2. Call 911 - which is the local emergency number.
3. Notify the NIOSH physician on-site.
4. Follow basic CPR and first-aid guidelines.
5. Notify one of the study field coordinators
   Field coordinators (Mary Schubauer-Berigan, NIOSH Division of Surveillance, Hazard Evaluations, and Field Studies, 4676 Columbia Parkway R15, Cincinnati, OH 45226, office: 513.841.4251; cell: 513.417.9486 or Matthew Dahm, Division of Surveillance, Hazard Evaluations, and Field Studies, 4676 Columbia Parkway R14, Cincinnati, OH 45226, office: 513.458.7136; cell: 618.560.0076).
6. Notify the facility Health and Safety coordinator.
6. If appropriate, arrange transportation to a local emergency care facility (will be specified when shop and clinic locations are known).

After the emergency has been handled, immediately call the following individuals:

Mary Schubauer-Berigan, Ph.D.
NIOSH Division of Surveillance, Hazard Evaluations, and Field Studies
4676 Columbia Parkway R15
Cincinnati, OH 45226
(513) 841-4251

or

Douglas Trout, M.D., M.P.H
NIOSH Division of Surveillance, Hazard Evaluations, and Field Studies
4676 Columbia Parkway R12
Cincinnati, OH 45226
(513) 841-4558
Information sheet from NIOSH Epidemiological Study: Clinical Examination Results

For employee:

Weight: ________________ lb
Height: ________________ inches
Waist circumference: ________________ inches
Average blood pressure: __________ (systolic)
____________________ (diastolic)
Average heart rate (pulse): ________________ (beats per minute)

GUIDANCE:

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP 120-139 or Diastolic BP 80-89 (Pre hypertension)</td>
<td>Confirm within 2 months</td>
</tr>
<tr>
<td></td>
<td>Evaluate within 1 week if TOD/CCD/DM*</td>
</tr>
<tr>
<td>Systolic BP 140-159 or Diastolic BP 90-99</td>
<td>Evaluate within 1 month</td>
</tr>
<tr>
<td></td>
<td>Evaluate within 1 week if TOD/CCD/DM*</td>
</tr>
<tr>
<td>Systolic BP &gt;160-179 or Diastolic BP &gt;100-109</td>
<td>Evaluate within 1 week</td>
</tr>
<tr>
<td>Systolic BP &gt;180 or Diastolic BP ≥110</td>
<td>Immediate treatment</td>
</tr>
<tr>
<td></td>
<td>Refer to private physician or an urgent care facility</td>
</tr>
</tbody>
</table>

Normal Blood Pressure
Systolic blood pressure < 120
Diastolic Blood Pressure < 80

Normal Pulse 60-100 beats per minute

*Target Organ Damage / Clinical Cardiovascular Disease/Diabetes Mellitus (TOD/CCD/DM)

- Heart disease
- Stroke or mini stroke TIA
- Kidney damage
- Poor leg circulation
- Damage to the retina of the eye
Dear Mr. (Ms.) (last name):

Thank you again for taking part in the National Institute for Occupational Safety and Health (NIOSH) Study of U.S. Workers Exposed to Carbon Nanotubes and Nanofibers. During our recent visit to (company name) on (date range), we conducted a number of assessments for our study, including (choose the consented and completed evaluations from among the following list) body measurements, blood pressure and heart rate measurements, spirometry, a complete blood count, and collection of specimens (blood and sputum) for future analysis of other biomarkers.

This letter includes your personal results from the tests that have direct clinical (medical) meaning for you and your doctor (choose the consented and completed evaluations from among the following list): the body measurements, blood pressure and heart rate, the complete blood count, and the spirometry. It also contains some information to help you interpret these results.

I. Body Measurements

Your measured height is: ___ inches

Your measured weight is: ___ pounds

Your measured waist circumference is: ___ inches

Your calculated body mass index (BMI) is: ______

The standard weight status categories associated with BMI ranges for adults are shown in the table below:

<table>
<thead>
<tr>
<th>BMI</th>
<th>Weight status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 18.5</td>
<td>Underweight</td>
</tr>
<tr>
<td>18.5 – 24.9</td>
<td>Normal</td>
</tr>
<tr>
<td>25.0 – 29.9</td>
<td>Overweight</td>
</tr>
</tbody>
</table>
Choose 1:

(For BMI below 18.5): Your BMI indicates that your weight is in the Underweight category for adults of your height.

(For BMI 18.5-24.9): Your BMI indicates that your weight is in the Normal category for adults of your height.

(For BMI 25.0-29.9): Your BMI indicates that your weight is in the Overweight category for adults of your height.

(For BMI 30.0 and above): Your BMI indicates that your weight is in the Obese category for adults of your height.

Maintaining a healthy weight may reduce the risk of chronic diseases associated with overweight and obesity.

II. Blood Pressure and Heart Rate Measurements

Your average blood pressure is: _________ (systolic)

___________(diastolic)

Normal blood pressure is 120/80.

Choose 1:

(For systolic BP <120 and diastolic BP <80): Your blood pressure is within the normal range.

(For systolic BP 120-139 or diastolic BP 80-89): Your blood pressure is elevated. We recommended that you share your results with your personal physician and undergo a medical evaluation within 2 months or sooner.

(For systolic BP 140-159 or diastolic BP 90-99): Your blood pressure is elevated. We recommended you share your results with your personal physician and undergo a medical evaluation within 1 month or sooner.

(For systolic BP >160-179 or diastolic BP >100-109): Your blood pressure is elevated. We recommended you share your results with your personal physician and undergo a medical evaluation within 1 week or sooner.

(For systolic BP >180 or diastolic BP >110): Your blood pressure was elevated. During our site visit we recommended immediate treatment and referred you to an urgent care facility.

Your average heart rate is: _____ beats per minute. Normal heart rate is 60–100 beats per minute.

Choose 1:

(For heart rate < 60): Your heart rate was below the lower limit of normal. We recommend you share this result and discuss its importance with your personal physician.

(For heart rate 60-100): Your heart rate was within the normal range.
(For heart rate > 100): Your heart rate was above the lower limit of normal. We recommend you share this result and discuss its importance with your personal physician.

**III. Complete blood count**

The attached report contains the results of your complete blood count. A complete blood count is typically not a definitive diagnostic test. Results outside the normal range may or may not require follow-up so it is important to share these results with your personal physician. Your physician may need to evaluate the results along with results of other blood tests, or additional tests may be necessary to determine next steps.

The table below contains your personal results and the laboratory reference ranges of the 4 clinically most important tests in your complete blood count. White blood cells help fight infection. Hemoglobin is the oxygen-carrying protein in red blood cells. Hematocrit is the proportion of red blood cells to the fluid component, or plasma, in your blood. Platelets help with blood clotting.

<table>
<thead>
<tr>
<th>Test name</th>
<th>Your result</th>
<th>Laboratory reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count</td>
<td>3.8–10.8 thousand/µL</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Choose 1: 13.2–17.1 g/dL (males) or 11.7–15.5 g/dL (females)</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>Choose 1: 38.5–50.0% (males) or 35.0–45.0% (females)</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>140–400 thousand/µL</td>
<td></td>
</tr>
</tbody>
</table>

**WBC:**

**Choose 1:**

(For WBC < 3.8 or > 10.8): Your white blood cell count is outside the laboratory reference range. This may be caused by a medical condition or a side effect of a medication. We recommend you share this result and discuss its importance with your personal physician within one month.

(For WBC 3.8-10.8): Your white blood cell count is within the laboratory reference range.

**Hemoglobin and hematocrit:**

**Choose 1:**

(For Hb or Hct outside the normal reference range): Your hemoglobin and/or hematocrit are outside the laboratory reference range. This may be caused by a medical condition or a side effect of a medication. We recommend you share this result and discuss its importance with your personal physician within one month.

(For Hb 13.2-17.1 and Hct 38.5-50 [males] or Hb 11.7-15.5 and Hct 35.0-45.0 [females]): Your hemoglobin and hematocrit are within the laboratory reference range.
Platelet count:

**Choose 1:**

*(For Pl < 140 or > 400):* Your platelet count is outside the laboratory reference range. This may be caused by a medical condition or a side effect of a medication. We recommend you share this result and discuss its importance with your personal physician within one month.

*(For Pl 140-400):* Your platelet count is within the laboratory reference range.

*(Text to be used if other values in the complete blood count are outside the laboratory reference range):* As you may notice on your complete results, one or more of the other values on your blood test were outside the laboratory reference range. These tests cannot be evaluated without a more thorough clinical evaluation than we were able to conduct for our study. Therefore, we recommend you share this result and discuss its importance with your personal physician within one month.

**IV. Spirometry**

The purpose of the coached breathing test (known as spirometry) is to determine how your lung function compares to expected normal lung function. The test includes measurements of the forced vital capacity (FVC) (this is the maximal or total amount of air you can forcefully breathe out after taking a deep breath) and the one-second forced expiratory volume (FEV₁) (this is the amount of air that you can breathe out in the first second of exhaling), and the calculation of the ratio of FEV₁ to FVC.

The results of your spirometry test were `{SpirometryInterp}`.

*{SpirometryInterp} =

(1) within normal limits.

(2) interpreted as having an obstructive abnormality.

(3) interpreted as having a restrictive abnormality.

(4) interpreted as having a mixed abnormality.

(5) not interpretable.

In the enclosed report entitled "Report of Spirometry Findings", your test results are compared to predicted values for a healthy, non-smoking person of the same age, height, sex, and race. We recommend you share this report with your personal physician so that it may be added to your medical records. Any abnormal test results should not be considered a diagnosis of disease; that determination can only be made by your personal physician following a complete medical evaluation, *(following phrase to be included for those with options 2-5 below)* which we recommend within the next two months. A graph of your breathing tests appears on the third page of the report. [SPIROMETRY OPTIONS]

[SPIROMETRY OPTIONS]

(1) Your lung function was within normal limits.
(2) An obstructive abnormality indicates that air is exhaled from the lungs more slowly than normal. This can be seen in certain lung conditions such as asthma, bronchitis, or emphysema. The greater the obstruction (the lower the FEV₁), the more difficult it is to exhale the air from the lungs.

(3) A restrictive abnormality indicates that the amount of air exhaled is smaller than normal. This can be seen in certain lung conditions such as lung scarring or fibrosis, or in people who are considerably overweight. It can also be seen in people who have a severe obstructive abnormality. The greater the restriction (the lower the FVC), the greater will be the possible physical limitation.

(4) A mixed abnormality is the combination of obstructive and restrictive abnormalities. It indicates that air is exhaled from the lungs more slowly than normal and the amount of air exhaled is also smaller than normal. This can be seen in people who have a severe obstructive abnormality.

(5) Unfortunately, the tests were not performed in an adequate manner for us to be able to interpret your test results. In part, this may represent a failure on our part to properly train you to perform this test. We recommend that you share these results with your physician and ask him or her whether you should have the spirometry test repeated.

Choose 1:
(For those who did not consent to having results sent to their personal physician) Your individual results have been sent only to you.
(For those who consented to having results sent to their personal physician) Your individual results have been sent to you and, at your request, to your personal physician.

Your individual results are important because they contribute to our evaluation of occupational exposure to carbon nanotubes or nanofibers. Only the grouped results for all worksites that participated in the study will be shared with your workplace.

We encourage you to discuss all of your test results with your personal physician. If you or your physician has any questions about these results or our study, please feel to contact me at 513-841-4116, or the study Principal Investigator, Mary Schubauer-Berigan, PhD, at 513-841-4251.

Sincerely yours,

Marie A. de Perio, MD
Medical Officer
Division of Surveillance, Hazard Evaluations and Field Studies

Enclosures
Dear Mr. (Ms.) (last name):

Thank you again for taking part in the National Institute for Occupational Safety and Health (NIOSH) Study of US Workers Exposed to Carbon Nanotubes and Nanofibers. During the NIOSH visit to (company name) on (date range), we collected a number of air samples over your entire work shift to determine your exposure to carbon nanotubes or nanofibers. The air samples, worn by you, were collected on one or two separate days. We also collected samples of carbon nanotubes or nanofibers that may have been on the skin of your wrists and hands. This letter includes your personal results from those samples.

**What we did**

We collected three air samples, worn by you on each day, to determine your exposure. The air samples included two samples to measure elemental carbon, which is a marker for carbon nanotube exposure, at two different sizes (inhaled and respirable). Inhalable particles are less than about 100 micrometers (µm) in size and when these particles are breathed in, they can deposit in the nose, mouth, windpipe (trachea), and the upper portions of the lung. Respirable particles are less than about 4 µm in size and when they are breathed in, they can enter the deepest parts of the lung, the alveoli. One air sample and one skin sample were also analyzed by microscope for visual evidence of carbon nanotubes in the air and on your palm and wrist.

**How we determine if exposures are acceptable**

Occupational exposure limits (OELs) have been developed by federal agencies such as NIOSH, the Occupational Safety and Health Administration (OSHA), and other safety and health organizations such as the American Conference of Governmental Industrial Hygienists (ACGIH®) to prevent harmful health effects from workplace exposures. OELs established by OSHA are enforced by law.

Currently, NIOSH is the only U.S. organization with an OEL for carbon nanotubes and nanofibers. The NIOSH OEL is $1 \text{ microgram per cubic meter} (\mu g/m^3)$ of elemental carbon at the respirable particle size. In instances where there are no established OELs, such as for elemental carbon at the inhalable particle size or the microscopy samples, we have compared

---

1 Note to NIOSH IRB: The currently published NIOSH draft REL is 7 \mu g/m³, but the draft currently under final review by the NIOSH Director has been reduced to 1 \mu g/m³. In each notification letter, we will use the current published NIOSH value.
your exposure to the average exposures of your co-workers at your workplace. We have also compared your exposure to the average exposures of other workers at different companies, performing similar work as you, from whom NIOSH has previously collected air samples.

**What we found in air for elemental carbon**

*Respirable Mass*
Your air levels of elemental carbon at the respirable particle size were \( X \ \mu g/m^3 \) and \( X \ \mu g/m^3 \). These samples were collected over your entire work shift. The air levels were (choose 1: above/below) the OEL of \( x^a \ \mu g/m^3 \) established by NIOSH.

*Inhalable Mass*
Your air levels of elemental carbon at the inhalable particle size were \( X \ \mu g/m^3 \) and \( X \ \mu g/m^3 \). The air levels were (choose 1: above/below) the average concentration of \( X \ \mu g/m^3 \) for co-workers at your workplace. The air levels were also (choose 1: above/below) the average concentration of \( X \ \mu g/m^3 \) collected from other workers at different companies performing similar work as you.

*Microscopy Air Samples*
Your air levels of visual carbon nanotubes and carbon nanotube clusters (agglomerates) were \( X \ \text{CNT Structures/m}^3 \) and \( X \ \text{CNT Structures/m}^3 \). These samples were collected over your entire work shift. The air levels were (choose 1: above/below) the average concentration of \( X \ \text{CNT Structures/m}^3 \) for co-workers at your workplace. The air levels were also (choose 1: above/below) the average concentration of \( X \ \text{CNT Structures/m}^3 \) collected from other workers at different companies performing similar work as you.

*Microscopy Skin Samples*
We took a sample from your palm and wrist at the end of your shift. The laboratory results of this sample confirmed the (choose 1: presence/absence) of carbon nanotubes on your palm and the (choose 1: presence/absence) of carbon nanotubes on your wrist.

**Summary**
A full report will be sent to the company contact at (company name) which summarizes all of our results. This report will not mention you by name. The report will also contain a discussion of what the exposure sampling means in terms of possible health effects along with our recommendations on how to reduce your exposures and better protect employees at your workplace. We encourage you to discuss your work and possible exposures with your healthcare providers. If you have any questions, please call me at (513) 458-7136 or email me at mdahm@cdc.gov.

Sincerely Yours,

Matthew Dahm, MPH 
Research Industrial Hygienist 
4676 Columbia Parkway, MS R-14 
Cincinnati, OH 45226 
O: 513-458-7136 
F: 513-841-4486 
E: mdahm@cdc.gov