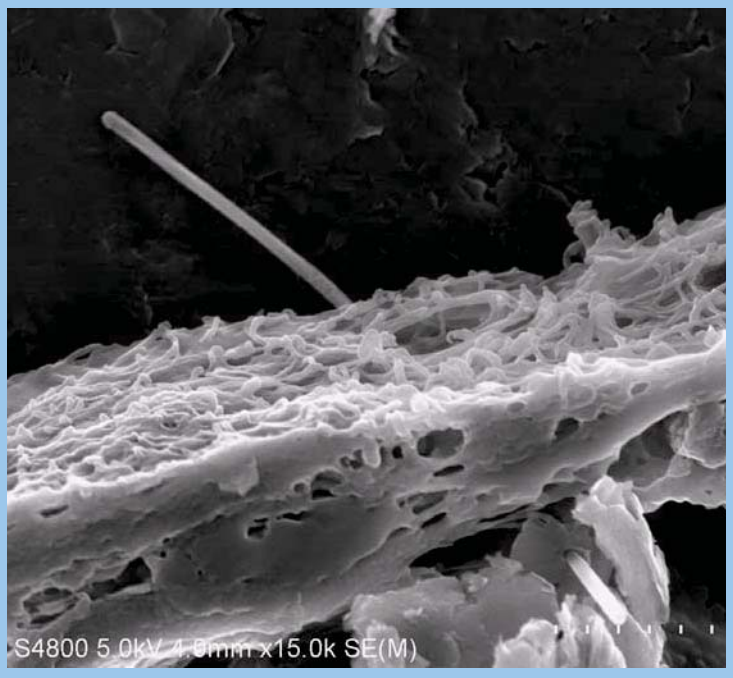
November 2010 Draft

NIOSH Current Intelligence Bulletin

Occupational Exposure to Carbon Nanotubes and Nanofibers



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

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DHHS (NIOSH) Publication No. 2010–XXX

Date: December 2010

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On the Cover: A field emission scanning electron micrograph of a multi-walled carbon nanotube (MWCNT) penetrating the pleura of the lung. *Image courtesy of Robert Mercer, and Diane Schwegler-Berry, NIOSH.*

Foreword

Executive Summary

Background

Currently there are no studies reported in the literature of adverse health effects in workers producing or using carbon nanotubes (CNT) or carbon nanofibers (CNF). The concern about worker exposure to CNT or CNF arises from results of animal studies. Several studies in rodents have shown: (1) an equal or greater potency of CNT compared to other inhaled particles known to be hazardous to exposed workers (ultrafine carbon black, crystalline silica, and asbestos) in causing adverse lung effects including pulmonary inflammation and fibrosis [Shvedova et al. 2005; Muller et al. 2005]; (2) the early onset and persistence of pulmonary fibrosis observed in CNT-exposed animals in short-term and subchronic studies [Shvedova et al. 2005, 2008; Porter et al. 2010; Pauluhn 2010a]; and (3) the reduced lung clearance in rats exposed to low mass concentrations of CNT [Pauluhn 2010a]. Findings of acute pulmonary inflammation and interstitial fibrosis have also been observed in mice exposed to CNF [Kisin et al. 2010]. In addition, the long and thin structure of some CNT and CNF dimensionally resemble asbestos fibers, and multi-walled carbon nanotubes (MWCNT) have been observed to migrate from pulmonary alveoli to the pleura [Hubbs et al. 2009; Porter et al. 2010; Mercer et al. 2010] tissue which is the same site in which malignant mesothelioma can develop due to asbestos exposure. Animal studies have also shown asbestos-type pathology associated with exposure to longer, straighter CNT structures [Poland et al. 2008; Takagi et al. 2008], and *in vitro* cell studies have shown that single-walled carbon nanotubes (SWCNT) can cause genotoxicity and abnormal chromosome number due to interference with mitosis (cell division) [Sargent et al. 2009]. Mesothelial tumors have been reported in a susceptible strain of mice after intraperitoneal injection of longer MWCNT (10-20 μm in length) [Takagi et al. 2008] but not by short MWCNT (<1 μm in length) [Muller et al. 2009]. Some evidence indicates that CNT with certain metals (nickel, 26%) and with higher metal content (17.7% vs. 0.2%Fe) are more toxic and fibrogenic [Lam et al. 2004; Shvedova et al.

2005, 2008]. However, both unpurified and purified (low metal content) CNT were associated with early-onset and persistent pulmonary fibrosis and other adverse lung effects. Though additional research is needed to further elucidate the mechanisms of biological responses to CNT and CNF, these findings of adverse respiratory effects in animals indicate the need for precautionary measures to limit the risk of occupational lung diseases in workers with potential exposure to CNT and CNF.

CNT and CNF are currently used in numerous industrial and biomedical applications, including electronics, lithium-ion batteries, solar cells, super capacitors, reinforced plastics, micro-fabrication conjugated polymer activators, biosensors, enhanced electron-scanning microscopy imaging techniques, and in pharmaceutical/biomedical devices for bone grafting, tissue repair, drug delivery, and medical diagnostics. CNT and CNF can be encountered in facilities ranging from research laboratories and production plants to operations where CNT and CNF are processed, used, disposed, or recycled. The extent of worker exposure to CNT and CNF is poorly understood, but workplace exposure measurements of CNT [Han et al. 2008; Bello et al. 2008, 2009; Tsai et al. 2009; Lee et al. 2010] and CNF [Methner et al. 2007; Evans et al. 2010] indicate the potential for worker exposure.

Assessment of the Health Risk and Recommended Exposure Limit

The dose-response data for CNT in animal studies provide a scientific basis for developing a recommended exposure limit (REL) to protect workers' health. The pulmonary responses of pulmonary inflammation and fibrosis observed in animals are relevant to occupational lung diseases associated with worker exposure to inhaled particles and fibers in the workplace. Although these observed adverse lung responses among exposed animals have persisted in subchronic studies, there is little evidence to evaluate whether these effects are associated with functional deficits in animals or whether they necessarily

correspond to effects that would result in functional deficits or be clinically significant among workers. The REL is based on the available subchronic and short-term animal dose-response data of early-stage fibrotic and inflammatory lung responses to CNT exposure. Benchmark dose (BMD) estimates from the animal data (and the 95% lower confidence limit estimates of the BMD) have been extrapolated to humans by accounting for species differences in alveolar lung surface area. Working lifetime exposure concentrations have been calculated based on estimates of either the deposited or retained alveolar lung dose of CNT assuming an 8-hour time-weighted average (TWA) work shift exposure during a 40-hour work week, 50 weeks per year, for 45 years.

In this risk analysis, NIOSH has determined that workers may be at risk of developing adverse respiratory health effects if exposed for a working lifetime at the upper limit of quantitation (LOQ) of NIOSH Method 5040, currently the recommended analytical method for measuring airborne CNT. The LOQ for NIOSH Method 5040 is 7 $\mu g/m^3$. Specifically, the animal data-based risk estimates indicate that workers may have >10% excess risk of developing early-stage pulmonary fibrosis if exposed over a full working lifetime at the upper LOQ for NIOSH Method 5040. Until improved sampling and analytical methods can be developed, and until data become available to determine if an alternative exposure metric to mass may be more biologically relevant, NIOSH is recommending a REL of 7 $\mu g/m^3$ elemental carbon (EC) as an 8-hr TWA respirable mass airborne concentration. Occupational exposures to all types of CNT can be quantified by NIOSH Method 5040 as airborne EC. While data from animal studies with CNF are more limited [Kisin et al. 2010], physiochemical similarities between CNT and CNF and findings of acute pulmonary inflammation and interstitial fibrosis in animals exposed to CNF indicate the need to also control occupational exposure to CNF at the REL using Method 5040.

Although the REL is set at the lowest airborne CNT and CNF concentration that can be accurately measured by NIOSH 5040 (i.e., LOQ of Method 5040), an

excess risk of adverse lung effects is predicted below this level. Therefore, efforts should be made to reduce airborne concentrations of CNT and CNF as low as possible below the REL. The value of 7µg/m^3 is a high estimate of the LOQ. For a given workplace assessment of exposures, a lower LOQ may be possible (see Appendix C). Further, the detection of exposures between the limit of detection (LOD) and LOQ are statistically significant and therefore, can be used to confirm the presence of airborne CNT or CNF (as opposed to non-detectable).

CNT are widely accepted to be durable due to the process they undergo during synthesis in which contaminating catalytic metals are frequently removed either by high temperature vaporization or acid treatment. Neither treatment is found to significantly alter the physical structure of CNT. Findings from animal studies also indicate that some types of CNT are biopersistent [Shvedova et al. 2005; Mercer et al. 2008; Pauluhn 2010b] as evident from impaired clearance of CNT from the lungs of rats and mice. Some types of CNT were shown in animal studies to be more potent (earlier onset and greater adverse lung response at a given mass dose) than other types of particles and fibers examined in those studies [Lam et al. 2004; Shvedova et al. 2005; Muller et al. 2005]. Other studies have shown that the size (e.g., length) of MWCNT and SWCNT may have an effect on their biological activity [Takagi et al. 2008; Poland et al. 2008; Muller et al. 2009]. These data indicate that exposure metrics other than airborne mass concentration (e.g., number concentration of CNT or CNF structures of specified dimensions) may be a better predictor of certain lung diseases (e.g., fibrosis).

Currently, the adverse pulmonary effects observed in animal studies are based on a mass dose and the NIOSH REL is also based on the mass (respirable) of CNT and CNF collected during air sampling (Method 5040). Given the low density and small diameters of individual CNT and CNF structures, a mass-based sampling method may not be sufficiently sensitive to detect all CNT and CNF structures in the air at low mass concentrations. Thus, research is needed to determine the most sensitive dose metrics for estimating various health risks

of exposures to CNT and CNF and to develop sampling and analytical methods corresponding to those metrics.

In the meantime, a respirable mass-based REL provides a means to identify job tasks with potential exposures to CNT and CNF and to ensure that appropriate measures are taken to limit worker exposure. If future studies associate health hazards with the total number of CNT and CNF structures or with the number of structures with specified dimensions, additional guidance, such as count-based exposure limits and improved sampling and analytical methods, may need to be developed.

Recommendations

Until results from research studies can fully elucidate the physicochemical properties of CNT and CNF that define their inhalation toxicity, steps should be taken to minimize CNT and CNF exposures of all workers and to implement an occupational health surveillance program that includes elements of hazard and medical surveillance. NIOSH recommends that employers and workers take the following steps to minimize potential health risks associated with exposure to CNT and CNF.

1. Recommendations for employers

- Use available information to continually assess current hazard potential related to CNT and CNF exposures in the workplace and make appropriate changes (e.g., sampling and analysis, exposure control) to protect workers health.

- Identify and characterize processes and job tasks where workers come in contact with bulk (“free-form”) CNT and CNT-containing materials (e.g., composites).

- When possible, substitute a non-hazardous or less hazardous material for CNT and CNF when feasible. When substitution is not possible, use engineering controls as the primary method for minimizing worker exposure to CNT and CNF.

- Establish criteria and procedures for selecting, installing, and evaluating the performance of engineering controls to ensure proper operating conditions. Make sure workers are trained on how to check and use exposure controls (e.g., exhaust ventilation systems).

- Routinely evaluate airborne exposures to ensure that control measures are working properly and that worker exposures are being maintained below the NIOSH REL of 7.0 µg/m^3 using NIOSH Method 5040 or an equivalent method (see Chapter 6 and Appendix C).

- Follow exposure and hazard assessment procedures for determining the need for and selection of proper personal protective equipment, such as clothing, gloves, and respirators (see Chapter 6).

- Educate workers on the sources and job tasks that may expose them to CNT and CNF and train them on how to use appropriate controls, work practices, and personal protective equipment to minimize exposure.

- Provide facilities for hand-washing and encourage workers to make use of these facilities before eating, smoking, or leaving the worksite.

- Provide facilities for showering and changing clothes, with separate facilities for storage of non-work clothing, to prevent the inadvertent cross-contamination of non-work areas (including take-home contamination).

- Use light-colored gloves, lab coats, and work bench surfaces to facilitate observation of contamination by dark CNT and CNF.

- Develop and implement procedures to deal with clean-up of CNT and CNF spills and de-contamination of surfaces.

- When respirators are provided for worker protection, the OSHA respiratory protection standard [29 CFR 1910.134] requires that a respiratory protection program be established that includes the following elements:

    - a medical evaluation of the worker's ability to perform the work while wearing a respirator,
    - regular training of personnel,
    - periodic workplace exposure monitoring,
    - respirator fit testing, and
    - respirator maintenance, inspection, cleaning, and storage.

1.1 Medical screening and surveillance

The evidence summarized in this document leads to the conclusion that workers occupationally exposed to CNT and CNF may be at risk of adverse respiratory effects. These workers may benefit from inclusion in a medical screening program recommended as a prudent means to help protect their health (see Figure 1):

1.1.1 Worker participation

Workers who could receive the greatest benefit from medical screening include:

a) Workers exposed to concentrations of CNT or CNF in excess of the REL (i.e., all workers exposed to airborne CNT or CNF at concentrations above 7 µg/m^3 elemental carbon as an 8-hr TWA), or

b) Workers in areas or jobs, activities, or processes involving contact with CNT or CNF who have the potential for intermittent elevated airborne concentrations to CNT or CNF (i.e., workers involved in the transfer, weighing, blending, or mixing of bulk CNT or CNF, or the cutting or grinding of composite materials containing CNT or CNF, or workers in areas where such activities are carried out by others, are at risk of being exposed).

1.1.2 Program oversight

Oversight of the medical surveillance program should be assigned to a qualified health care professional who is informed and knowledgeable about potential workplace exposures, routes of exposure, and potential health effects related to CNT and CNF.

1.1.3 Screening elements

Initial evaluation

- An initial (baseline) e**v**aluation should be conducted by a qualified health care professional and should consist of:
  - an occupational and medical history
  - a physical examination with an emphasis on the respiratory system
  - a spirometry test (Anyone administering spirometry testing as part of the medical screening program should have completed a NIOSH-approved training course in spirometry or other equivalent training.)
  - a chest X-ray (All chest X-ray images should be interpreted by a NIOSH-certified B Reader using the standard International Classification of Radiographs

of Pneumoconiosis [ILO 2000 or the most recent equivalent].)

- other examinations or medical tests deemed appropriate by the responsible health care professional (The need for specific medical tests may be based on factors such as abnormal findings on initial examination.)

Periodic evaluations

- Periodic evaluations should be conducted at regular intervals (e.g., annual) or at other times (e.g., post-incident) as deemed appropriate by the responsible health care professional based on data gathered in the initial evaluation, ongoing work history, changes in symptoms such as new, worsening, or persistent respiratory symptoms, and when process changes occur in the workplace (e.g., a change in how CNT or CNF are manufactured or used or an unintentional "spill"). Evaluations should include:
  - a respiratory symptom update
  - an occupational and medical history update
  - a physical examination
  - consideration of specific medical tests (e.g., spirometry, chest X-ray)

Written reports of medical findings

- The health care professional should give each worker a written report containing:
  - The individual worker's medical examination results.
  - Medical opinion(s) and/or recommendation(s) concerning any relationship(s) between the individual worker's medical

condition(s) and occupational exposure(s), any special instructions on the individual's exposure(s) and/or use of personal protective equipment, and any further evaluation or treatment.

- For each examined employee, the health care professional should give the employer a written report specifying:
  - Any work or exposure restrictions based on the results of medical evaluations.
  - Any recommendations concerning use of personal protective equipment.
  - A medical opinion as to whether any of the worker's medical condition(s) is likely to have been caused or aggravated by occupational exposures.
- Findings from the medical evaluations having no bearing on the worker's ability to work with CNT or CNF should not be included in any reports to employers. Confidentiality of the worker's medical records should be enforced in accordance with all applicable regulations and guidelines.

1.1.4 Worker training

Worker training should include information sufficient to allow workers to understand the nature of potential workplace exposures, potential health risks, routes of exposure, and instructions for reporting health symptoms. Workers should be provided with information about the purposes of medical screening, the health benefits of the program, and the procedures involved.

1.1.5 Periodic evaluation of data and screening program

- Standardized medical screening data should be periodically aggregated and evaluated to identify patterns of worker health that may be linked to

work activities and practices that require additional primary prevention efforts. This analysis should be performed by a qualified health professional or other knowledgeable person to identify patterns of worker health that may be linked to work activities or exposures. Confidentiality of worker's medical records should be enforced in accordance with all applicable regulations and guidelines.

- Employers should periodically evaluate the elements of the medical screening program to ensure that the program is consistent with current knowledge related to exposures and health effects associated with occupational exposure to CNT and CNF.

Other important components related to occupational health surveillance programs, including medical surveillance and screening, are discussed in Appendix B.

2. Recommendations for workers

- Ask your supervisor for training on how to protect yourself from the potential hazards associated with your job, including exposure to CNT and CNF.
- Know and use the exposure control devices and work practices that keep CNT and CNF out of the air and off your skin.
- Understand when and how to wear a respirator and other personal protective equipment (such as gloves, clothing, eye wear) that your employer might provide.
- Avoid handling CNT and CNF in a '*free particle'* state (e.g., powder form).
- Store CNT and CNF, whether suspended in liquids or in a powder form, in closed (tightly sealed) containers whenever possible.

- Clean work areas at the end of each work shift (at a minimum) using either a HEPA-filtered vacuum cleaner or wet wiping methods. Dry sweeping or air hoses should not be used to clean work areas.

- Do not store or consume food or beverages in workplaces where bulk CNT or CNF or where CNT- or CNF-containing materials are handled.

- Prevent the inadvertent contamination of non-work areas (including take-home contamination) by showering and changing into clean clothes at the end of each work day.

Contents

1 Introduction

Many nanoscale-based products are now commercially available. These include nanoscale powders, solutions, and suspensions of nanoscale materials, as well as composite materials and devices incorporating nanomaterials. The International Organization for Standardization (ISO) has developed nomenclature and terminology for nanomaterials [ISO/TS 2008]. According to ISO 27687:2008, a *nano-object* is defined as material with one, two, or three external dimensions in the size range from approximately 1-100 nanometers (nm). Sub-categories of a nano-object are (1) *nanoplate,* a nano-object with one external dimension at the nanoscale; (2) *nanofiber,* a nano-object with two external dimensions at the nanoscale with a nanotube defined as a hollow nanofiber and a nanorod as a solid nanofiber; and (3) *nanoparticle,* a nano-object with all three external dimensions at the nanoscale. Nano-objects are commonly incorporated in a larger matrix or substrate referred to as a *nanomaterial.*

Carbon nanotubes (CNT) are nanoscale cylinders of carbon (essentially consisting of seamlessly 'rolled' sheets of graphene) that can be produced with very large aspect ratios. There is no single type of carbon nanotube. They may differ in shape, dimension, physical characteristics, surface coatings, chemical composition ('raw' CNT, which contain residual metal catalysts vs. 'purified' CNT, from which the metal catalysts have been removed) or surface functionalization. Single-walled carbon nanotubes (SWCNT) consist of a single rolled graphene sheet and have a typical diameter of approximately 1 nm. Multi-walled carbon nanotubes (MWCNT) consist of many single-walled tubes stacked one inside the other with diameters in the range of 2-100 nm. SWCNT and MWCNT can vary in length with some being up to many tens of micrometers long [Thostenson et al. 2001]. Carbon nanofibers (CNF), which are structurally similar to MWCNT, have typical diameters on the order of 40 to 200 nm [Ku et al. 2006]. CNF have lengths ranging from tens of micrometers to several centimeters, average aspect ratios of

>100, and display various morphologies, including cupped or stacked graphene structures. The primary characteristic that distinguishes CNF from CNT resides in graphene plane alignment. If the graphene plane and fiber axis do not align, the structure is defined as CNF, but when parallel, the structure is considered a CNT [ISO/TS 2008].

A growing body of literature indicates a potential hazard from exposure to various types of carbon nanotubes and nanofibers. A number of studies in rodents have shown adverse lung effects at relatively low mass doses of CNT (Tables 2a,b,c and 3a,b,c), including pulmonary inflammation and rapidly developing, persistent fibrosis. Similar effects have been recently observed with exposure to CNF (Table 3b). It is not known how universal these adverse effects are, i.e., whether they occur in animals exposed to all types of CNT and CNF, and whether they occur in additional animal models. Most importantly, it is not yet known whether similar adverse health effects occur in humans following exposure to CNT or CNF.

Because of their small size, structure, and low surface charge, CNT and CNF can be difficult to separate in the bulk form and tend to be agglomerated or to agglomerate quickly when released in the air, which can affect their potential to be inhaled and deposited in the lungs. The extent to which workers are exposed to CNT and CNF in the form of agglomerates or as single tubes or structures is unclear due to limited exposure measurement data.

This Current Intelligence Bulletin (CIB) summarizes the adverse respiratory health effects that have been observed in laboratory animal studies with SWCNT, MWCNT, and CNF. A recommended exposure limit (REL) for CNT and CNF is given to help minimize the risk to workers for developing respiratory disease and to assist in determining the need for exposure control measures and the implementation of an occupational health surveillance program.

2 Potential for Exposure

There has been extensive research on the novel application of CNT and CNF due to their unique physical and chemical properties. CNT and CNF are mechanically strong, flexible, lightweight, heat resistant, and have high electrical conductivity [Walters et al. 1999; Yu et al. 2000]. The commercial market for CNT and CNF is estimated to grow substantially over the next decade [Lux Research 2007]. Carbon nanotubes and nanofibers are currently used in a variety of applications including: electronics, lithium-ion batteries, solar cells, super capacitors, reinforced plastics, micro-fabrication conjugated polymer activators, biosensors, enhanced electron/scanning microscopy imaging techniques, and in pharmaceutical/biomedical devices for bone grafting, tissue repair, drug delivery, and medical diagnostics [E nanonewsletter 2008]. Of biological relevance, CNT and CNF are poorly soluble, although functionalization and surface treatment may influence their ability to be degraded in biological systems [Kagan et al. 2010].

There is currently limited information on the number of workers potentially exposed to CNT and CNF. However, it has been projected that nanotechnology will employ millions of workers worldwide within the next decade [Roco 2004].

There is also limited information on sources of CNT and CNF exposure with much of the reported workplace exposure data coming from research laboratories manufacturing and handling CNT- and CNT-composite materials and from small industrial operations synthesizing CNT or CNF. However, there is the potential for worker exposure throughout the life cycle of CNT- and CNF-product use (processing, use, disposal, recycling) and in a variety of workplace settings from research laboratories to production facilities [Maynard and Kuempel 2005]. The extent of exposure in those workplace settings has not been well characterized. Airborne exposures to CNT and CNF can occur during the transfer, weighing, blending, and mixing of the bulk powders, or during the cutting

of CNT- and CNF-composite materials, especially when no control measures are in place.

2.1 Exposure to carbon nanotubes

Recent assessments of airborne exposure to MWCNT in a research laboratory manufacturing and handling MWCNT found total-particulate concentrations ranging from 37 µg/m^3 (weighing operation) to 430 µg/m^3 (blending process) in the absence of exposure controls [Han et al. 2008]; the implementation of engineering controls (e.g., ventilated enclosure of MWCNT blending process) significantly reduced airborne particulate concentrations, often to non-detectable concentrations. Transmission electron microscopy (TEM) analysis (NIOSH Method 7402) performed on personal and area samples collected during the blending of MWCNT found airborne concentrations ranging from 172.9 tubes/cm^3 (area) to 193.6 tubes/cm^3 (personal). Airborne MWCNT concentrations were significantly reduced to 0.018 - 0.05 tubes/cm^3 after enclosing and ventilating the furnace and the blending process. The diameter and length of the tubes in the personal and area samples were 52-56 nm and 1473-1760 nm (avg. 1.5 µm), respectively. Maynard et al. [2004] also assessed the propensity for aerosol particles to be released during the agitation of unprocessed SWCNT material in a laboratory-based study and during the handling (e.g., furnace removal, powder transfer, cleaning) of unrefined material at four SWCNT production facilities in which laser ablation and high-pressure carbon monoxide techniques were used to produce SWCNT. Particle measurements taken during the agitation of unprocessed material in the laboratory indicated the initial airborne release of material (some visually apparent) with the particle concentration of the aerosol (particles <0.5µm in diameter) observed to decrease rapidly over time. With no agitation, particles around 0.1µm in diameter appeared to be released from the SWCNT material, probably as a result of the airflow across the powder. At the four production facilities, short-term SWCNT mass concentrations were estimated (using a catalyst metal as surrogate) to range from 0.7 to 53 µg/m^3 (area samples) in the absence of exposure controls. When samples were

evaluated by scanning electron microscopy (SEM), most of the aerosolized SWCNT were observed to be agglomerated, with agglomerated sizes typically larger than 1 µm.

Potential particle and MWCNT exposure concentrations were determined by Bello et al. [2008] during chemical vapor deposition (CVD), growth, and subsequent handling of vertically-aligned carbon nanotube films. Continuous airborne particle measurements were made using a real-time fast mobility particle sizer (FMPS) and a condensation particle counter (CPC) throughout the furnace operation. No increase in total airborne particle concentration (compared to background) was observed during the removal of MWCNT from the reactor furnace or during the detachment of MWCNT from the growth substrate (a process whereby MWCNT are removed from the substrate with a razor blade). Electron microscopic analysis of a personal breathing zone sample (PBZ) collected on the furnace operator found no detectable quantity of MWCNT, either as individual tubes or as agglomerates. No mention was made on the use of engineering controls (e.g., local exhaust ventilation, fume hood) to prevent exposure to MWCNT.

In a study designed to investigate the release of CNT during the dry and wet cutting of CNT-composite materials, airborne samples were collected to determine particle number, respirable mass, and nanotube concentrations [Bello et al. 2009]. Two different composites containing MWCNT (10-20 nm diameters) were cut using a band-saw or rotary cutting wheel. The laboratory study was designed to simulate the industrial cutting of CNT-composites. Personal breathing zone (PBZ) and area samples (close to the emission source) were collected during dry cutting (without emission controls), and during wet cutting (equipped with a protective guard surrounding the rotary cutting wheel). The cutting of composite materials ranged from 1 to 3 minutes. The dry-cutting of composite materials generated statistically significant ($p<0.05$) quantities of airborne nanoscale and fine particles when compared to background particle concentrations. Although the particle number concentration was dominated by

the nanoscale and fine fractions, 71 to 89% of the total particle surface area was dominated by the respirable (1-10µm) aerosol fraction. Reported mean PM_{10} mass concentrations for area and PBZ samples were 2.11 and 8.38 mg/m^3, and 0.8 and 2.4 mg/m^3, respectively, during the dry cutting of composites. Submicron and respirable fibers were generated from dry cutting of all composites. TEM analysis of samples found concentrations of 1.6 to 3.8 fibers/cm^3 (area samples) during the cutting of CNT-alumina and base-carbon composite materials, respectively. A PBZ fiber concentration of 0.2 fibers/cm^3 was observed during the dry cutting of base-alumina composite materials. No fiber measurement data were reported for the wet cutting of composite materials. No increase in mean PM_{10} mass concentrations were observed in 2 of 3 area samples collected during the wet cutting of composites. In the third sample, the observed high particle concentration was attributed to extensive damage of the protective guard around the rotary cutting wheel.

The potential for airborne particle and SWCNT and MWCNT release was evaluated in a laboratory setting in which both types of CNT were produced using CVD [Tsai et al. 2009]. A quantitative assessment (i.e., morphology, aerosol size) of the exposure was made during the synthesis of SWCNT and MWCNT in which modifications of the production methods were made to ascertain how changes in the production of CNT influenced aerosol particle size and concentration (e.g., SWCNT synthesis with and without a catalyst; growth of MWCNT on a substrate and with no substrate). An FMPS and an aerodynamic particle sizer (APS) were used to monitor aerosol particle size and concentrations. Background particle concentrations were determined to assist in quantifying the release of SWCNT and MWCNT during their synthesis and handling. Samples were also collected for analysis by TEM to determine particle morphology and elemental composition. Particle measurements made inside a fume hood during the synthesis of SWCNT were found to be as high as 10^7particles/cm^3 with an average particle diameter of 50 nm; PBZ samples collected outside of the fume hood were considerably lower (< 2000 particles/cm^3). The difference between

particle concentrations obtained during SWCNT growth using a catalyst and the control data (no catalyst) was small and was postulated to be a result of particles being released from the reactor walls of the furnace even when no SWCNT were being manufactured. Particle measurements made during the synthesis of MWCNT were found to peak at 4 x10^6 particles/cm^3 when measured inside the fume hood. Particle size ranged from 25-100 nm when a substrate was used for MWCNT growth and from 20-200 nm when no substrate was present. Airborne particle concentrations and particle size were found to vary as a result of the temperature of the reactor with higher particle concentrations and smaller particle sizes observed at higher temperatures. PBZ samples collected outside of the fume hood during MWCNT synthesis found particle concentrations similar to background particle concentrations. TEM analysis of MWCNT samples indicated the presence of individual particles as small as 20 nm with particle agglomerates as large as 300 nm. Some individual MWCNT were observed but were often accompanied by clusters of carbon and iron particles. The diameters of the tubes were reported to be about 50 nm. The use of a fume hood that was extra wide and high and operated at a constant velocity of 0.7 m/s face velocity appeared to be effective in minimizing the generation of turbulent airflow at the hood face which contributed to the good performance of the fume hood in capturing the airborne release of SWCNT and MWCNT during their synthesis.

Lee et al. [2010] investigated the potential airborne release of MWCNT at 7 facilities (e.g., research laboratories, industries) where MWCNT was either being synthesized by CVD or handled (e.g., ultrasonic dispersion, spraying). Real-time aerosol monitoring was conducted using a scanning mobility particle sizer (SMPS) and a CPC to determine particle size and concentration. Personal and area samples were also collected for determining mass concentrations (total suspended particulate matter) and for TEM (NIOSH Method 7402) and SEM analysis for particle identification and characterization. Background measurements of nanoparticle exposure were conducted at 2 of the 7 worksites prior to starting work to assist in establishing a baseline for airborne nanoparticle

concentrations. Most of the handling of MWCNT during synthesis and application was performed inside a laboratory fume hood where most of the exposure measurements were made. Exposure concentrations of total suspended particulate matter ranged from 0.0078 to 0.3208 mg/m^3 and 0.0126 to 0.1873 mg/m^3 for personal and area samples, respectively. TEM and SEM analysis of filter samples found no detectable amounts of MWCNT but only aggregates of metal particles such as iron and aluminum that were used as catalysts in the synthesis of MWCNT. The highest airborne particle releases were observed in area samples collected during catalyst preparation (18,600-75,000 particles/cm^3 for 20-30 nm diameter particles) and the opening of the CVD reactor (6,974-16,857 particles/cm^3 for 20-50 nm diameter particles). Other handling processes such as CNT preparation, ultrasonic dispersion, and opening the CNT spray cover also generated the release of nanoparticles. The ultrasonic dispersion of CNT generated particles in the range of 120 to 300 nm which were larger in size than those released from other processes.

The release of airborne carbon-based nanomaterials (CNMs) was investigated during the transfer and ultrasonic dispersion of MWCNT (10-20 nm diameters), fullerenes, and carbon black (15 nm diameter) inside a laboratory fume hood with the air flow turned off and the sash halfway open [Johnson et al. 2010]. Exposures were assessed during the weighing and transferring of dry CNMs to beakers filled with reconstituted freshwater, and sonicated in deionized water and reconstituted freshwater with and without natural organic matter. The study was designed to determine the relative magnitude of airborne nanomaterial emissions associated with tasks and materials used in the evaluation of environmentally relevant matrices (e.g., rivers, ponds, reservoirs). Direct reading real-time instruments (i.e., CPC, optical particle counter) were used to determine airborne particle number concentrations with the results compared to particle number concentrations determined from general air samples collected in the laboratory before and after the laboratory process. Samples were also collected for TEM analysis to verify the presence of CNMs. Airborne particle number concentrations

for all evaluated tasks exceeded background particle concentrations which were inversely related to particle size with the size distribution of particles skewed toward those CNMs with an aerodynamic diameter < 1 µm. Airborne particle number concentrations for MWCNT and carbon black during the sonication of water samples were significantly higher than those found during the weighing and transferring of dry CNMs. TEM analysis of airborne area samples revealed agglomerates of all CNMs with MWCNT agglomerates observed to be 500 to 1,000 nm in diameter.

There is evidence that workers exposed to the dust following the collapse of the World Trade Center (WTC) in 2001 were possibly exposed to CNT as a result of the high temperatures generated during the WTC disaster caused by the combustion of fuel in the presence of carbon and metals [Wu et al. 2010]. Some persons who developed severe respiratory impairment were found to have interstitial lung disease consistent with small airways disease, bronchiolocentric parenchyma disease, and non-necrotizing granulomatous conditions. Lung tissue analysis by transmission electron microscopy (TEM) showed variable amounts of aluminum and magnesium silicates, chrysotile asbestos, calcium phosphate and calcium sulfate, shards of glass, and CNT of various size dimensions. However, it remains unclear whether the presence of CNT in the lung had any role in causing the lung pathology.

2.2 Exposure to carbon nanofibers

Some research has been conducted to date on workplace emissions or exposure to carbon nanofibers (CNF) [Methner et al. 2007; Evans et al. 2010]. In a NIOSH health hazard evaluation conducted at a university-based research laboratory, the potential release of airborne CNF was observed at various processes using real-time aerosol instruments (e.g., CPC, ELPI, aerosol photometer) [Methner et al. 2007]. General area exposure measurements indicated slight increases in airborne particle number and mass concentrations relative to background measurements (outdoors and offices) during the transfer of CNF prior to

weighing and mixing, and during the chopping and wet saw cutting of a polymer composite material. Airborne total carbon mass concentrations (per NIOSH Method 5040) within the laboratory processing area were 2 to 64 times higher than those of a nearby office area with the highest peak exposure concentration (1094 μg/m^3) found during the wet saw cutting of the CNF composite material. No indoor particle concentrations exceeded the outdoor background concentrations. Particles having a diameter of about 400 nm or greater were found in greater number during wet-saw cutting, while the number of particles having a diameter of about 500 nm or greater were elevated during the weighing and mixing of CNF. Airborne samples collected directly on TEM grids were analyzed for the presence of CNF. Some fibers observed by TEM had diameters larger than the 100 nm criterion used to define a nanofiber, which was consistent with results reported by Ku et al. [2006] in which the mobility diameter of aerosolized CNF was observed to be larger than 60 nm with a modal aerodynamic diameter of about 700 nm. The majority of CNF observed by TEM were loosely agglomerated, rather than single fibers, which was in general agreement with the particle size measurements made by real-time instruments.

Detailed investigations of exposures at different job tasks were conducted at a facility manufacturing and processing CNF [Evans et al. 2010] in which CNF production totaled 14,000 kg $year^{-}$. Various types of direct-reading instruments (e.g., CPC, ELPI, photometer/w cyclone, diffusion charger, fast particulate size spectrometer (FPSS)) and respirable particle mass concentrations were used to assess CNT exposures and to evaluate instrument performance. A transient increase in respirable mass concentration was observed during manual bagging of the final product and was attributed to aerosolized CNF. The tamping of the bag to settle contents and subsequent closing dispersed CNF through the bag opening into the workplace. High particle number and active surface area concentrations were found during the opening of the dryer and the manual redistribution of CNF product that attributed to the presence of ultrafine particles emitted from the dryer and as by-products formed through the high-temperature

thermal processing of CNF. No elevations in respirable mass concentrations were observed during these operations suggesting that significant quantities of CNF were not released into the workplace. However, the transfer or dumping of dried CNF from a dryer to a drum, and subsequent bag change-out of final product, contributed the largest transient increases in respirable mass concentrations with concentrations during these events exceeding 1.1 and 0.5 mg/m^{-3}, respectively. The authors concluded that integrated particle number and active surface area concentrations (i.e., using CPC and diffusion charger) were not useful in assessing the contribution of emissions from CNF in the workplace since measurements were dominated by ultrafine particle emissions. Respirable particle mass concentrations estimated by the photometer appeared to be the most useful and practical metric for measuring CNF when using direct reading instruments. Furthermore, TEM analysis of size selective area samples indicated that large fiber bundles were present, further supporting findings that particle mass concentrations may be a more practical metric for monitoring CNF exposures.

3 Evidence for Potential Adverse Health Effects

Various types of laboratory animal studies have been conducted with CNT using different routes of exposure to evaluate potential toxicity (Tables 1-3). These studies have shown a consistent toxicological response (e.g., pulmonary inflammation, fibrosis) independent of the study design (intratracheal, aspiration, and inhalation). Exposure to SWCNT and MWCNT are of special concern because of their small size and fiber-like dimensions. Their nanometer diameters and micrometer lengths closely resemble the dimensions of some mineral fibers (e.g., asbestos). Results from laboratory animal studies with SWCNT, MWCNT, and CNF show similar pulmonary responses as those reported for some respirable particles and durable fibers. In some studies, CNT-induced lung

fibrosis developed more rapidly and at a lower mass burden than either ultrafine carbon black or quartz [Lam et al. 2004; Shvedova et al. 2005; Ryman-Rasmussen et al. 2009b]. Unpurified CNT can also contain residual metal catalysts that are left over from the manufacturing process. In a biological environment, these metal catalysts can promote the generation of reactive oxygen species, thereby, enhancing the potential for cytotoxicity [Pulskamp et al. 2007; Barillet et al. 2010].

CNT are widely accepted to be durable due to the process they undergo during synthesis in which contaminating catalytic metals are frequently removed either by high temperature vaporization or acid treatment. Neither treatment is found to significantly degrade the physical structure of CNT. Kagan et al. [2010] reported that *in vitro* myeloperoxidase, which is found in high concentrations in polymorphonuclear neutrophils (PMN), degraded SWCNT. However, it is uncertain as to whether PMN-derived myeloperoxidase would degrade SWCNT *in vivo* (e.g., in the lung) because: 1) PMN recruitment after SWCNT exposure is a transient rather than persistent response, 2) there is no strong evidence for SWCNT phagocytosis by PMN, and 3) SWCNT are found in the lungs of animals 1-year after pharyngeal aspiration [Shvedova et al. 2005; Mercer et al. 2008]. Several animal studies have also shown that the size (e.g., length) of MWCNT and SWCNT may have an effect on their biological activity [Takagi et al. 2008; Poland et al. 2008; Muller et al. 2009]. Intraperitoneal injection of long MWCNT (> 5 μm) caused fibrotic peritoneal adhesions and peritoneal tumors [Takagi et al. 2008] and granulomaous lesions [Poland et al. 2008] similar to that observed in crocidolite asbestos-treated animals. In contrast, when animals were exposed to short MWCNT (<1μm length) by intraperitoneal injection [Poland et al. 2008; Muller et al. 2009] only acute inflammation was observed.

The physicochemical properties (e.g., dimension, composition, surface characteristics) of CNT and CNF can be modified to reflect their intended commercial use. In addition, CNT can be functionalized, thus changing their surface chemistry. Toxicological effects of such changes remain largely

unexplored except for some limited evidence indicating that structural defects [Muller et al. 2008a; Fenoglio et al. 2008], surface modification [Sayes et al. 2006], and nitrogen doping [Carrero-Sanchez et al. 2006] of CNT can alter their toxicity potential.

Discrete nanoparticles have one dimension of < 100 nm. However, when nanoparticles, including CNT and CNF, are suspended in test media, agglomerates of nanoparticles of various sizes frequently occur. This is particularly evident in test media used in recent studies where animals have been exposed to CNT suspensions by intratracheal instillation (ITI), intraperitoneal injection, or by pharyngeal aspiration (a technique where particle deposition closely resembles inhalation). Agglomerate size for CNT and CNF is normally smaller in a dry aerosol than when suspended in physiological media. Evidence from toxicity studies in laboratory animals indicates that decreasing agglomerate size increases the pulmonary response to exposure [Shvedova et al. 2007, 2008; Mercer et al. 2008]. The extent to which agglomerates of CNT and CNF de-agglomerate in biological systems (e.g., in the lung) is still unknown.

Differences in results from animal studies have been attributed to differences in physicochemical properties, surface area, the degree of agglomeration of the test material, and differences in the observation period following termination of exposure [McDonald and Mitchell 2008; Lison and Muller 2008].

3.1 Single-walled carbon nanotubes (SWCNT)

Mice or rats exposed to SWCNT by ITI or pharyngeal aspiration have developed granulomatous lesions at sites in the lung where agglomerates of SWCNT deposited [Lam et al. 2004; Warheit et al. 2004; Shvedova et al. 2005; Mangum et al. 2006; Mercer et al. 2008]. Lam et al. [2004] investigated the toxicity of SWCNT obtained from 3 different sources each with different amounts of residual catalytic metals being present. Mice were exposed by ITI to three different types of SWCNT (containing either 27% Fe, 2% Fe, or 26% Ni and 5% Y) at concentrations of 0.1 or 0.5 mg, to carbon black (0.5 mg), or to quartz (0.5 mg)

and toxicologically assessed 7 or 90 days post exposure. All types of SWCNT studied produced persistent epithelioid granulomas (which were associated with particle agglomerates) and interstitial inflammation that were dose related. No granulomas were observed in mice exposed to carbon black and only mild to moderate inflammation of the lungs was observed in the quartz exposure group. High mortality (5/9 mice) occurred within 4 to 7 days in mice instilled with the 0.5 mg dose of SWCNT containing nickel and yttrium). Warheit et al. [2004] exposed rats via ITI to concentrations of 1 or 5 mg/kg SWCNT, quartz, carbonyl iron, or graphite particles and evaluated effects 24-h,1-week, 1-month, and 3-months post exposure. The SWCNT were reported to have nominal diameters of 1.4 nm and lengths >1μm that tended to agglomerate into larger size structures. In this study, ~15% of the SWCNT-instilled rats died within 24 hours of SWCNT exposure, apparently due to SWCNT blockage of the upper airways. In the remaining rats, a transient inflammatory response of the lung (observed up to 1-month post exposure) and a non-dose dependent series of multifocal granulomas that were non-uniform in distribution were observed. Only rats exposed to quartz developed a dose-dependent lung inflammatory response that was observed at 3-months. Exposures to carbonyl iron or graphite particles produced no significant adverse effects.

Progressive interstitial fibrosis of alveolar walls has also been reported in mice when exposed via pharyngeal aspiration to SWCNT at doses of 10, 20, 40 μg/mouse [Shvedova et al. 2005]. As with studies by Lam et al. [2004] and Warheit et al. [2004], epitheloid granulomas were observed at deposition sites of SWCNT aggregates. This granuloma formation was rapid (within 7 days) and dose dependent and persisted over the 60-day post exposure period. A rapid, dose-dependent, and progressive development of interstitial fibrosis in pulmonary regions distant from deposition sites of SWCNT agglomerates was observed and appeared to be associated with deposition of dispersed SWCNT structures. These finding were consistent with those reported by Magnum et al. [2006] in which rats exposed to 2 mg/kg via pharyngeal aspiration developed granulomas

at sites of SWCNT agglomerates and diffuse interstitial fibrosis at 21-days post exposure. When a more dispersed delivery of SWCNT was given by aspiration to mice (10 µg) an accelerated increase in collagen production in the alveolar interstitium occurred that progressed in the absence of persistent inflammation, with the development of few granulomatous lesions [Mercer et al. 2008]. A significant submicrometer fraction of the dispersed SWCNT was observed to rapidly migrate into alveolar interstitial spaces with relatively little of the material being a target for macrophage engulfment and phagocytosis.

Shvedova et al. [2008] compared the responses resulting from exposure via pharyngeal aspiration [Shvedova et al. 2005] against exposure via inhalation of more-dispersed SWCNT [Baron et al. 2008]. One set of mice were exposed by inhalation to 5 mg/m^3, 5 h/day for 4 days, while mice exposed by aspiration were given a single dose of 5, 10, or 20 µg. The SWCNT for both studies had dimensions of 0.8-1.2 nm diameters and 100-1,000 nm lengths with a measured surface area (Brunauer-Emmett-Teller method [BET]) of 508 m^2/g. Both studies reported acute lung inflammation followed by the development of granulomatous pneumonia and persistent interstitial fibrosis; these effects were observed for both purified (0.2% Fe) [Shvedova et al. 2005] and unpurified (17.7% Fe) [Shvedova et al. 2008] SWCNT. The finding that the acute lung inflammation resolved after the end of exposure while the pulmonary fibrotic response persisted or progressed is unusual compared to lung responses observed from other inhaled particles; the findings indicate that the mechanism may involve the direct stimulation of fibroblasts by dispersed SWCNT that were translocated to the lung interstitium [Wang et al. 2010]. Quantitatively, mice exposed by inhalation (dispersed SWCNT) were 4-fold more prone to the development of an inflammatory response, interstitial collagen deposition, and fibrosis, when compared (at an estimated equivalent lung dose) to mice exposed by aspiration to poorly dispersed SWCNT. Mice exposed by inhalation to 5 mg/m^3 SWCNT [Shvedova et al. 2008] is relevant, since the Occupational Safety and Health

Administration (OSHA) permissible exposure limit (PEL) for graphite of 5 mg/m^3 is often used for controlling workplace exposures to CNT.

3.2 Multi-walled carbon nanotubes (MWCNT)

Exposures to well-dispersed MWCNT in mice via pharyngeal aspiration have resulted in dose- and time-dependent pulmonary inflammation, as well as central nervous system effects, at doses ranging from 10 to 80 µg/mouse [Sriram et al. 2007; Sriram et al. 2009; Porter et al. 2010; Wolfarth et al. 2009; Hubbs et al. 2009].

Exposure to MWCNT in mice at doses of 10, 20, 40 or 80 µg resulted in acute pulmonary inflammation and damage, granulomas, and pulmonary fibrosis occurring 7 days post-exposure and persisting through 56 days post-exposure [Porter et al. 2010]. There was also evidence that MWCNT can reach the pleura [Porter et al. 2010] and that alveolar macrophages containing MWCNT can migrate to the lymphatics and cause lymphatic inflammation [Hubbs et al. 2009]. Some of the MWCNT (mean diameter of 49 nm and mean length of 4.2 µm) were observed penetrating the outer lung wall into the intrapleura space [Hubbs et al. 2009; Mercer et al. 2010].

Lung inflammation and fibrosis have also been observed in rats when exposed by ITI to long (5.9 µm) or short (0.7 µm) MWCNT at doses of 0.5, 2 or 5 mg of either ground or unground MWCNT [Muller et al. 2005] and examined up to 60 days post-exposure. Rats that received ground MWCNT showed greater dispersion in the lungs, and fibrotic lesions were observed in the deep lungs (alveolar region). In rats treated with unground MWCNT fibrosis appeared mainly in the airways rather than in the lungs. The biopersistence of the unground MWCNT was greater than that of the ground MWCNT (81% vs. 36 %). At an equal mass dose, ground MWCNT produced a similar inflammatory and fibrogenic response as chrysotile asbestos and a greater response than ultrafine carbon black [Muller et al. 2005]. Similar acute lung effects have been reported in guinea pigs at doses of 12.5 mg [Grubek-Jaworska et al. 2005] and 15 mg

[Huczko et al. 2005], in mice exposed by ITI at a dose of 0.05 mg (average diameter of 50 nm, average length of 10 µm) [Li et al. 2007], or in rats [Liu et al. 2008] at doses of 1, 3, 5 or 7 mg (diameters of 40 to 60 nm, lengths of 0.5 to 5 µm). In contrast, Elgrabli et al. [2008a] reported cell death but no histopathological lesions or fibrosis in rats exposed by ITI at doses of 1, 10 or 100 µg MWCNT (diameters of 20 to 50 nm, lengths of 0.5 to 2 µm).

Several short-term inhalation studies using mice or rats have been conducted to assess the pulmonary [Mitchell et al. 2007; Arkema 2008; Ma-Hock et al. 2009; Porter et al. 2009; Ryman-Rasmussen et al. 2009b; Pauluhn 2010a] and systemic immune effects [Mitchell et al. 2007] from exposure to MWCNT. Mitchell et al. [2007] reported the results of a whole body short-term inhalation study with mice exposed to MWCNT (diameters of 10 to 20 nm, lengths of 5 to15 µm) at concentrations of 0.3, 1 or 5 mg/m^3 for 7 or 14 days (6 h/day) (although there was some question regarding whether these structures were actually MWCNT [Lison and Muller 2008]). Histopathology of lungs of exposed animals showed alveolar macrophages containing black particles; however, there was no observed inflammation or tissue damage. Systemic immunosuppression was observed after 14 days, although without a clear concentration-response relationship. Mitchell et al. [2009] reported that the immunosuppression mechanism of MWCNT appears to involve a signal originating in the lungs that activates cyclooxygenase enzymes in the spleen. Porter et al. [2009] reported significant pulmonary inflammation and damage in mice 1 day after inhalation of well dispersed MWCNT (10 mg/m^3, 5 h/day, 2-12 days; mass aerodynamic diameter of 1.3 µm, count aerodynamic diameter of 0.4 µm). In addition, granulomas were also observed encapsulating MWCNT in the terminal bronchial/proximal alveolar region of the lung. In an inhalation (nose-only) study with mice exposed to 30 mg/m^3 MWCNT (lengths of 0.5 to 50 µm) for 6 hours, a high incidence (9 of 10 mice) of fibrotic lesions occurred [Ryman-Rasmussen et al. 2009b]. MWCNT were found in the subpleural region of the lung 1 day post exposure with sub-pleural fibrosis occurring at 2 weeks post exposure that

progressed through 6 weeks of follow-up. No fibrosis was observed in mice exposed to 1 mg/m^3 of MWCNT or in mice exposed to 30 mg/m^3 of nanoscale carbon black.

Subchronic inhalation studies with MWCNT have also been conducted in laboratory studies with rats to assess the potential dose-response and time course for developing pulmonary effects [Arkema 2008; Ma-Hock et al. 2009; Pauluhn 2010a]. Ma-Hock et al. [2009] reported on the results of a 90-day inhalation (head-nose) study with rats exposed at concentrations of 0.1, 0.5 or 2.5 mg/m^3 MWCNT (BASF Nanocyl NC 7000) for 6h/day, 5days/week for 13 weeks. No systemic toxicity was observed but exposure caused hyperplastic responses in the nasal cavity and upper airways (larynx and trachea), and granulomatous inflammation in the lung and in lung-associated lymph nodes at all exposure concentrations. The incidence and severity of the effects were concentration-related. No lung fibrosis was observed but pronounced alveolar lipoproteinosis did occur. Ellinger-Ziegelbauer and Pauluhn [2009] conducted a short-term inhalation bioassay (prior to the Pauluhn 2010a subchronic study) to investigate the dependence of pulmonary inflammation resulting from exposure to one type of MWCNT (Bayer Baytubes®) containing a small amount of cobalt (residual catalyst). Groups of rats were exposed to 11 mg/m^3 MWCNT containing either 0.53% or 0.12% cobalt to assess differences in pulmonary toxicity due to metal contamination. Another group of rats was exposed to 241 mg/m^3 MWCNT (0.53% cobalt) to serve the purpose of hazard identification. All animals were exposed to a single nose-only inhalation exposure of 6h followed by a post-exposure period of 3-months. Time course of MWCNT-related pulmonary toxicity was compared to rats exposed to quartz in post-exposure weeks 1, 4, and 13 to distinguish early, possibly surface area/activity-related effects from retention-related poorly soluble particle effects. Rats exposed to either quartz or MWCNT resulted in somewhat similar patterns of concentration dependent pulmonary inflammation during the early phase of the study. The pulmonary inflammation induced by quartz increased during the 3-months post-exposure period whereas

that induced by MWCNT regressed in a concentration-dependent manner. The time course of pulmonary inflammation associated with retained MWCNT was independent on the concentration of residual cobalt. Pauluhn [2010a] using the same MWCNT (0.53% cobalt) used in the study by Ellinger-Ziegelbauer and Pauluhn [2009] exposed rats (nose-only) at concentrations 0.1, 0.4, 1.5 and 6 mg/m^3 for 6h/day, 5days/week for 13 weeks to MWCNT that was described as being agglomerated (mean diameter of 3 µm). Lung clearance of MWCNT at the low doses was slow with a marked inhibition of clearance at 1.5 and 6 mg/m^3. Histopathology analysis at 6-months post exposure revealed exposure-related lesions in the upper respiratory (e.g., goblet cell hyper-and/or metaplasia) and lower respiratory (e.g., inflammation in the bronchiole-alveolar region) tract in animals exposed at concentrations of 0.4, 1.5 and 6 mg/m^3 as well as inflammatory changes in the distal nasal cavities that were similar to that found by Ma-Hock et al. [2009]. In rats exposed at 6 mg/m^3 a time-dependent increase of bronchiolio-alveolar hyperplasia was observed as well as changes in granulomas and an increase in collagen deposition that persisted through the 39-week post exposure observation period. No treatment related effects were reported for rats exposed at 0.1 mg/m^3. In a report submitted by Arkema [2008] to EPA, rats exposed (nose only) to agglomerates of MWCNT (Arkema) at concentrations of 0.1, 0.5, and 2.5 mg/m^3 for 6h/day for 5 days, histopathological effects were observed that were consistent with those reported by Ma-Hock et al. [2009], Ellinger-Ziegelbauer and Pauluhn [2009] and Pauluhn [2010a]. An increase of various cytokine and chemokines in the lung along with the development of granulomas were found in the 0.5 and 2.5 mg/m^3 exposure groups while no treatment related effects were reported at 0.1 mg/m^3.

Intraperitoneal injection studies in rodents have been frequently used as screening assays for potential mesotheliogenic activity in humans. To date, exposures to only a few fiber types are known to produce mesotheliomas in humans. These include the asbestos minerals and erionite fibers. Several animal studies [Takagi et al. 2008; Poland et al. 2008; Muller et al. 2009; Varga and

Szendi 2010] have been conducted to investigate the hazard potential of various sizes and doses of MWCNT and SWCNT to cause a carcinogenic response. Takagi et al. [2008] reported on the intraperitoneal injection of 3 mg of MWCNT in p53 +/- mice (genetically engineered mouse model) in which approximately 28% of the structures were > 5 µm in length and an average diameter of 100 nm. MWCNT-treated mice revealed moderate to severe fibrotic peritoneal adhesions, fibrotic peritoneal thickening, and a high incidence of macroscopic peritoneal tumors in 88% of treated mice after 25 weeks. Histological examination found mesothelial lesions in the vicinity of fibrosis and granulomas. Similar findings were also seen in the crocidolite asbestos-treated mice. Minimal mesothelial reactions and no mesotheliomas were produced by the same dose of (non-fibrous) C_{60} fullerene. Poland et al. [2008] reported that the peritoneal (abdominal) injection of long MWCNT but not short MWCNT induced inflammation and granulomaous lesions on the abdominal side of the diaphragm at one week post-exposure. In contrast to the Takagi et al. [2008] study, wild type mice were used and exposed to a much lower dose (50 µg) of MWCNT. Although this study documented acute inflammation, it did not evaluate whether this inflammation would persist and progress to mesothelioma. A lack of a carcinogenic response was reported by Muller et al. [2009] and Varga and Szendi [2010] in rats exposed to either MWCNT or SWCNT. No mesotheliomas were noted 2 years after intraperitoneal injection of MWCNT in rats at a single dose of 2 or 20 mg [Muller et al. 2009]. However, the MWCNT sample used in the study was very short (avg. < 1 µm in length) and the findings were consistent with the low biological activity observed in the Poland et al. [2008] study when mice were exposed to short MWCNT. Varga and Szendi [2010] reported on the implantation of either MWCNT or SWCNT in F-344 rats (six per group) at a dose of 10 mg (25 mg/kg bw). Gelatin capsules containing either SWCNT (<2 nm diameters x 4-15 µm lengths), MWCNT (10-30 nm diameters x 1-2 µm lengths), or crystalline zinc oxide (negative control) were implanted into the peritoneal. Histological examination at 12 months revealed only a granulomatous reaction of foreign body type with epithelioid and multinucleated giant cells in CNT exposed

animals. No information was reported on what effect the delivery of SWCNT and MWCNT in gelatin capsules had on their dispersion in the peritoneal given the tendency of CNT to agglomerate. If SWCNT and MWCNT remained agglomerated following delivery this may have resulted in the lack of a mesothelioma-inducing effect. The low biological activity observed for the short MWCNT sample (≤ 2 μm) used in the study was consistent with the findings from Poland et al. [2008] and Muller et al. [2009] in which short MWCNT were also used.

3.3. Carbon Nanofibers (CNF)

Recent observations indicate that exposure to CNF can cause respiratory effects similar to that observed in animals exposed to CNT [Kisin et al. 2010]. Results from mice exposed by aspiration to CNF at doses of 40 and 120 μg and evaluated post-exposure at 1, 7, and 28 days and at 1 year were compared to results from mice exposed to crocidolite asbestos (120 μg) or SWCNT (40 and 120 μg). Mice exposed to CNF developed acute inflammation and early onset of interstitial fibrosis and increased collagen deposition. However, mice exposed to 120 μg of CNF or asbestos exhibited less collagen deposition as compared to that seen in mice exposed to SWCNT at the same dose.

4 Conclusions-Hazard Assessment

Results of laboratory animal studies indicate a risk of acute lung inflammation, epithelioid granulomas (microscopic nodules), and rapidly developing fibrotic responses at relatively low mass doses of CNT and CNF (Chapter 3). A number of the studies have shown: 1) an equal or greater potency of CNT compared to other inhaled particles (ultrafine carbon black, crystalline silica, and asbestos) in causing adverse lung effects including pulmonary inflammation and fibrosis [Shvedova et al. 2005; Muller et al. 2005]; 2) the early onset and persistent

pulmonary fibrosis in CNT-exposed animals in short-term and subchronic studies [Shvedova et al. 2005, 2008; Porter et al. 2010; Pauluhn 2010a]; 3) similar pulmonary responses in animals (e.g., acute lung inflammation, interstitial fibrosis) exposed to purified and unpurified CNT [Shvedova et al. 2005; Shvedova et al. 2008] and 4) the reduced lung clearance in rats exposed to low mass concentrations of CNT [Pauluhn 2010a]. Findings of acute inflammation as well as interstitial fibrosis have also been observed in mice exposed to CNF [Kisin et al. 2010]. In addition, the long-thin structures of some CNT and CNF resemble asbestos, and MWCNT have been observed to migrate to the pleura [Hubbs et al. 2009; Porter et al. 2010; Mercer et al. 2010], the tissue in which mesothelioma can develop due to asbestos exposure. Animal studies have also shown asbestos-type pathology associated with the longer, straighter CNT structures [Poland et al. 2008; Takagi et al. 2008]. Mesothelial tumors have been reported in a susceptible strain of mice receiving intraperitoneal injection of longer MWCNT (10-20 μm in length) [Takagi et al. 2008]; whereas a chronic bioassay of a short MWCNT (<1 μm in length) did not produce mesothelioma [Muller et al. 2009]. Results from cellular studies have shown that SWCNT can cause genotoxicity and abnormal chromosome number due to interference with mitosis (cell division) [Sargent et al. 2009] and that metal contamination (unpurified SWCNT) plays a major role in cytotoxicity [Shvedova et al. 2003]. Currently, there are no studies reported in the literature on the adverse health effects in workers producing or using CNT or CNF. However, since humans can also develop lung inflammation and fibrosis in response to inhaled particles and fibers, it is reasonable to assume that at equivalent exposures (e.g., airborne respirable mass concentrations) to CNT and CNF, workers may also be at risk of developing these adverse lung effects.

Although data on workplace exposures to CNT and CNF are limited, aerosolization of CNT and CNF has been shown to occur at a number of operations during research, production, and use of CNT and CNF, including such work tasks as transferring, weighing, blending, and mixing. Worker exposure to

airborne CNT and CNF have frequently been observed to be task specific and short-term in duration with exposure concentrations (frequently reported as particle number or mass concentrations) found to exceed background exposure measurements when appropriate engineering controls are not used to reduce exposures [Maynard et al. 2004; Methner et al. 2007; Han et al. 2008; Bello et al. 2009; Tsai et al. 2009; Evans et al. 2010; Johnson et al. 2010; Lee et al. 2010]. Comprehensive workplace exposure evaluations are needed to characterize the extent of airborne exposure to CNT and CNF and to determine what control measures are the most effective in reducing worker exposures.

The findings of adverse respiratory effects (i.e., pulmonary fibrosis, granulomatous inflammation) in animals indicate the need for precautionary measures to reduce the health risk to workers exposed to CNT and CNF. Long-term inhalation studies are needed to determine whether CNT and CNF can cause cancer in laboratory animals at doses equivalent to potential workplace exposures. In addition, the potential for migration of CNT through the lungs to the mesothelium after inhalation requires further investigation. Until results from animal research studies can fully elucidate the mechanisms in which inhalation exposure to CNT and CNF cause adverse lung effects, all types of CNT and CNF should be considered an occupational respiratory hazard and the following actions should be taken to minimize health concerns:

1) minimize workplace exposures, and

2) establish an occupational health surveillance program for workers exposed to CNT and CNF.

5 CNT Risk Assessment and Recommended Exposure Limit

5.1 Risk assessment and recommended exposure limit (REL)

NIOSH bases its recommended exposure limits (RELs) on quantitative risk assessments when possible. Quantitative risk assessment provides estimates of the severity and likelihood of an adverse response associated with exposure to a hazardous substance. The hazard assessment based on the toxicology studies (Chapter 4) and the quantitative risk estimates derived from the risk analysis of the toxicological studies (Appendix A) provide the health basis for developing an occupational exposure limit for CNT and CNF. The technological feasibility of measuring worker exposures to airborne CNT and CNF in addition to the hazard and risk assessments, provide an additional basis for the REL. The establishment of health-based exposure limits is the first consideration by NIOSH in setting a REL, although the ability to measure the substance reliably in the workplace and the ability to control worker exposure are also important considerations in the establishment of the REL.

Several approaches were evaluated to derive occupational exposure limits (OELs) for MWCNT and SWCNT including the use of data from subchronic animal inhalation studies in which adverse lung effects were observed [Ma-Hock et al. 2009; Pauluhn 2010b]. NIOSH conducted a quantitative risk assessment using data from animal studies in which various types of CNT (i.e., MWCNT and SWCNT with different metal content) were observed to cause adverse lung effects (see Chapter 3 and Appendix A). Human-equivalent risk estimates were derived using benchmark dose methods applied to the animal dose-response data. In the absence of validated lung dosimetry models for CNT, lung doses were estimated assuming either deposited or retained lung dose in animals or humans. The findings from this analysis indicate that workers are potentially at risk of developing adverse lung effects (i.e., pulmonary fibrosis and granulomatous inflammation) if exposed to airborne CNT over a 45-year working

lifetime. Based on results from subchronic animal inhalation studies with MWCNT [Ma-Hock et al. 2009; Pauluhn 2010a], a working lifetime exposure of 0.2 – 2 μg/m^3 (8-hour TWA concentration) was estimated to be associated with a 10% excess risk of early-stage adverse lung effects (95% lower confidence limit estimates) (Appendix A, Table A-3). Risk estimates derived from other animal studies (e.g., single dose with 90-day follow-up) using SWCNT and other MWCNT (Appendix A, Tables A-3 and A-4) were supportive of these estimates.

There remains some uncertainty in extrapolating respiratory effects observed in short-term or subchronic animal studies to the potential for causing chronic respiratory effects in humans. Based on currently available data, it is difficult to assess the relative potency of the various types of CNT and CNF because there has been limited systematic study of multiple types of CNT and CNF using the same study design. These studies differ in factors including the rodent species and strain, the measure of adverse lung effects, and the exposure and post-exposure durations. However, even when these factors are taken into account, the findings consistently showed that CNT and CNF: 1) can be inhaled and retained in the lungs, 2) cause the early onset of pulmonary fibrosis following exposure to some CNT at an equal or greater extent than observed at the same mass dose of other inhaled particles or fibers examined in the same studies, and 3) the adverse lung responses can persist or progress after the end of the exposure (up to 6 months in the study by Pauluhn 2010a). Of particular concern is the finding that the pulmonary fibrotic effects were not reversible after exposure ended. In the absence of epidemiological studies, the animal studies provide the best available scientific data for risk assessment and REL development.

Despite differences in the type and composition of SWCNT and MWCNT used in animal studies, the risk estimates across the different types of CNT and studies are associated with relatively low mass exposure concentrations. While data from laboratory animal studies with CNF are limited, the similarities in physiochemical properties and adverse lung effects between CNF and CNT supports the need to

control exposures to CNF at the REL derived for CNT. NIOSH is recommending an occupational exposure limit for CNT and CNF to minimize the risk for developing adverse lung effects. A mass-based airborne exposure limit is being recommended because this exposure metric is the same used in determining the dose-response relationship in animal studies and deriving risk estimates, as well as the most common exposure metric used to date in monitoring work place exposures to CNT and CNF. While the desire is to establish an REL that would eliminate any potential risk for developing respiratory disease, limitations exist in reliably measuring airborne CNT and CNF using available mass-based sampling and analytical methods. NIOSH is recommending that NIOSH Method 5040 [NIOSH 2003; Birch 2004a,b] (or equivalent method) be used to measure work place airborne exposure to respirable CNT and CNF. The upper (high) estimate of the limit of quantification (LOQ) of Method 5040 is 7µg/m^3, as an 8-hr time-weighted average (TWA) concentration. NIOSH is recommending a REL of 7 µg/m^3 as an 8-hr TWA airborne respirable mass concentration for up to a 40-hr week. However, NIOSH recognizes that the REL may not be completely health protective but its use should help to lower the risk for developing lung disease and assist employers in establishing an occupational health surveillance program that includes elements of hazard and medical surveillance. Until improvements in sampling and analytical improvements can be made in measuring airborne exposures to CNT and CNF, continued efforts should be made to reduce airborne concentrations as low as possible below the REL by optimizing the sampling and analysis of exposures when possible (Appendix C). Based on available workplace exposure data, it is not possible for NIOSH to determine whether the NIOSH REL can be achieved in all workplaces where exposure to CNT and CNF occur; however, exposure data that has been reported indicate that the implementation of appropriate control measures (e.g., engineering, enclosures) can eliminate or greatly reduce worker exposures [Han et al. 2008; Tsai et al. 2009; Lee et al. 2010].

5.2 Other derived occupational exposure limits for CNT

One of the earliest OELs for CNT was proposed by the British Standards Institute [BSI 2007]; their "benchmark exposure limit" (BEL), was proposed at 0.1 fiber/cm^3, or one-tenth of their asbestos exposure limit (see Table 4). An interim OEL for MWCNT was also proposed in a report by the Japanese New Energy and Industrial Technology Development Organization (NEDO) [Kobayashi et al. 2009]. It was based on a NOAEL for pulmonary inflammation at 0.37 mg/m^3 in an unpublished 4-week inhalation study in rats. The equivalent rat lung dose rate was calculated to be 6.0 µg/kg/day [Kobayashi et al. 2009]. This value was then divided by an uncertainty factor of 2 for individual difference, resulting in 3.0 µg/kg/day, a value considered as an "acceptable MWCNT exposure to humans." This value was converted to 0.21 mg/m^3 (TWA, 8-hr/d, 5 d/wk), which was proposed as an "acceptable exposure concentration…in working environments." From this information, NIOSH calculates that 3.0 µg/kg/day in a 70 kg worker would result in a total daily dose of 210 µg. Assuming that a worker inhales 10 m^3 of air in an 8-hr day [ICRP 1994], this total daily dose would be attained at an 8-hr TWA concentration of 0.021 mg/m^3 (i.e., 21 µg/ m^3). Nanocyl [2009] derived an estimated OEL of 2.5 µg/m^3 for an 8-hr TWA exposure based on applying an overall assessment factor of 40 to the LOAEL of 0.1 mg/m^3 in the Ma-Hock et al. [2009] subchronic rat inhalation study with MWCNT.

Pauluhn [2010b] derived an occupational exposure limit OEL using subchronic data in rats inhaling MWCNTs (Baytubes®) [Pauluhn 2010a]. This approach was based on the biological mechanism of volumetric overloading of alveolar macrophage-mediated clearance of particles from the lungs of rats [Morrow 1988]. Increased particle retention half-time (an indication of clearance overload) was reported in rats exposed by subchronic inhalation to MWCNT (Baytubes®) at concentrations from 0.1 to 6 mg/m^3 [Pauluhn 2010a, b]. Lung retention half-times were greater for MWCNT (Baytubes®) compared to other particles at given mass doses [Pauluhn 2010b]. Benchmark concentration (BMC) estimates were calculated at 0.16 to 0.78 mg/m^3 for the pulmonary inflammation (PMN) and

fibrotic (collagen) responses; however, Pauluhn [2010b] selected the lower value of 0.1 mg/m^3 (NOAEL in rat subchronic study) to derive a human-equivalent concentration. The NOAEL was multiplied by a series of ratios to adjust for human and rat differences in various factors affecting the estimated particle lung dose (i.e., ventilation rate, pulmonary particle deposition and retention kinetics, and alveolar macrophage number and volume in each species). The combination of these ratios resulted in a final factor of 2, by which the rat NOAEL was divided, to arrive at a human-equivalent concentration of 0.05 mg/m^3 (8-hr TWA) as the OEL for MWCNT (Baytubes®). No uncertainty factors were used in deriving that estimate.

In any CNT risk assessment, an area of uncertainty concerns the estimation of the human-equivalent dose. In Kobayashi et al. [2009], the normalization of lung dose from rat to human based on equivalent dose per unit body weight does not account for species-specific differences in inhalation rate, lung surface area, or particle size-specific lung deposition fractions. In Pauluhn [2010b], the human lung retention of CNT is assumed to follow simple first-order clearance kinetics. First-order clearance has been shown to describe the rat lung retention of poorly soluble, low-toxicity particles at doses below overloading, whereas a reduced clearance rate coefficient is needed to describe particle retention at overloading doses in the rat [Tran et al. 1999; CIIT and RIVM 2006]. In humans, a simple first-order clearance model has been shown to under-predict the long-term particle burden in human lungs, even at low exposure concentrations [ICRP 1994; Kuempel et al. 2001; Bailey et al. 2008; Gregoratto et al. 2010]. Although human lung dosimetry models have not been evaluated or validated for CNT, it may be reasonable to assume in the absence of other data that the clearance of poorly-soluble CNT from the lungs will be no faster than observed for other poorly-soluble particles, and may be slower, as evidenced by impaired clearance of MWCNT (Baytubes®) even at low mass concentrations in rats [Pauluhn 2010a, b]. In the NIOSH risk assessment (Appendix A), rat and human lung dose

estimates include this range of possible CNT clearance mechanisms, from assuming normal particle clearance to assuming no clearance of deposited CNT.

Based on benchmark dose (BMD) modeling methods, the NIOSH risk assessment provides a standardized method for risk estimation. NOAEL-based approaches do not estimate risk but assume safe exposure or zero risk below the derived OEL. According to BMD-based estimates, excess risks of greater than 10% of early-stage adverse lung effects would be expected at the OELs based on the NOAEL approaches [Pauluhn 2010a; Kobayaski et al. 2009].

Although these CNT risk assessments used different methods and assumptions, the derived occupational exposure limits (OELs) are fairly similar relative to OELs for larger respirable particles. The currently proposed OELs for CNT range from 2.5 to 50 $\mu g/m^3$ (8-hr TWA concentration) [Nanocyl 2009; Kobayashi et al. 2009; Pauluhn 2010b], including the NIOSH REL of 7 $\mu g/m^3$. These CNT OELs are considerably lower than the current U.S. OELs for graphite or carbon black (approximately 2.5 to 5 mg/m^3), by a factor of 100 to 1000. This is relevant because in the absence of OELs for CNT, the OELs for graphite and carbon black are sometimes used on Material Safety Data Sheets (MSDS) as an exposure limit for CNT. Each of these CNT risk assessments supports the need to control exposures to CNT in the workplace to low airborne mass concentrations ($\mu g/m^3$) to protect workers' health, and to develop more sensitive methods to measure airborne concentrations of CNT.

6 Recommendations

In light of current scientific evidence concerning hazard potential from experimental animal studies, appropriate steps should be taken to minimize

workers exposure to CNT and CNF through the development and implementation of an exposure control strategy. Elements of that strategy should include:

1) the conduct of exposure assessments as part of an overall risk management and hazard surveillance program,

2) guidelines for selecting, installing and evaluating engineering controls (e.g., local exhaust ventilation, dust collection systems),

3) the education and training of workers in the use of good work practices in the handling of bulk CNT and CNF as well as CNT- and CNF-containing materials,

4) procedures for the selection and use of personal protective equipment (i.e., clothing, gloves. respirators),

5) the implementation of a medical surveillance program for workers potentially exposed to CNT or CNF with conduct of specific medical screening tests when warranted (Figure 1), and

6) the routine (e.g., annual) and systematic evaluation of worker exposure to CNT or CNF when there is a process change in how CNT or CNF are manufactured or handled.

6.1 Exposure assessment

NIOSH is recommending that a mass-based airborne concentration measurement be used for monitoring workplace exposures to all types of CNT and CNF until additional data are available to determine if other measurement metrics or techniques would be more effective in protecting workers' health. NIOSH is currently evaluating the efficacy of various sampling techniques for measuring CNT and CNF and may make additional recommendations at a later date.

Personal exposure concentrations to CNT and CNF should be determined as elemental carbon (EC) by NIOSH Method 5040 [NIOSH 1994; Birch 2004a,b] or an equivalent method. Measurement results from NIOSH Method 5040 should provide a reasonable estimate of worker's respirable exposure to CNT and CNF at the NIOSH REL of 7µg/m^3 8-hr TWA when the predominant workplace exposure to EC material is CNT or CNF. The REL of 7µg/m^3 is the estimated upper LOQ for Method 5040, but a lower LOQ can be obtained (see Appendix C). The use of high flow rate respirable samplers (cyclones) may help to provide a sufficient amount of sample to permit the measurement of CNT/CNF above the LOQ even for samples collected for less than a full work shift [Lee et al. 2010]. In work environments where exposure to other types of EC (e.g., diesel exhaust, combustion products) and fibrous particulates might occur, the use of additional analytical techniques can help to better characterize exposures. For example, analysis of airborne samples by transmission electron microscopy (TEM) equipped with x-ray energy dispersive spectroscopy (EDS), can help to verify the presence of CNT and CNF from other possible EC containing particles (e.g., diesel soot, carbon black). Consideration should also be given to using TEM to characterize the structures (e.g., shape, size) of aerosolized CNT and CNF. Collection of this information may be helpful should future efforts to control exposures be based on a number and size concentration of airborne CNT or CNF structures.

The evaluation of worker personal exposures to CNT and CNF should be a regular and systematic process that focuses on identifying sources of emissions and assessing the effectiveness of exposure controls. The collection of area (static) airborne samples for CNT and CNF can provide "activity pattern data" [Duan and Mage 1997] which can be used to better quantify the airborne release of CNT and CNF occurring at specific processes or job task activities. While these data can be useful for identifying possible causes of high exposure for remediation, these data are vulnerable to spatial variation in exposure concentrations due to systematic variations that occur in processes and handling

procedures and shouldn't be used in predicting worker exposures. NIOSH [NIOSH 2009a; Methner et al. 2010] and others [Brouwer 2009] have developed emission assessment guidance for determining the release of engineered nanoparticles that can be adapted for determining sources of exposure to CNT and CNF.

As part of the initial workplace hazard surveillance, NIOSH recommends identifying those workers with the highest potential for exposure to CNT and CNF [NIOSH 2009a], as well as the tasks and processes associated with those potential exposures. Performing targeted exposure sampling of workers involved in those tasks can be part of an overall exposure sampling strategy to protect workers' health. Although a specific sampling strategy has not been developed for evaluating workplace exposures to CNT and CNF, the same principles developed for the exposure measurement of other aerosols [e.g., NIOSH 1977; Leidel and Busch 1994] should apply to workers with potential exposure to CNT or CNF. When the goal of sampling is to determine whether or not worker exposures are being controlled below the REL, initial sampling efforts should focus on those workers thought to have the highest exposure concentrations (i.e., maximum risk worker) [NIOSH 1977; Leidel and Busch 1994]. This type of strategy may be more efficient and require fewer resources for identifying potential exposures above the REL, although periodic sampling of all workers or groups of workers (identified as having similar exposures) should also be performed. The periodic sampling will ensure that the targeted sampling groups include all workers with potential for exposures above the REL. In workplaces where the number of workers potentially exposed is small, consideration should be given to sampling all workers.

6.2 Engineering controls

If the CNT- or CNF-containing material cannot be substituted with a less hazardous or non-hazardous substance, then engineering controls should be installed and tailored to the process or job task to control worker exposure to

CNT and CNF based on hazard assessment. Because of limited published workplace exposure data for CNT and CNF, it's unknown whether worker respirable mass exposures can be maintained below the NIOSH REL of 7µg/m^3 as an 8-hour TWA at all workplaces. However, exposure control techniques such as source enclosure (i.e., isolating the generation source from the worker) and well designed local exhaust ventilation (LEV) systems equipped with high efficiency particulate air (HEPA) filters have be shown to be effective for capturing airborne nanoparticles including CNT and CNF [Old and Methner 2008; NIOSH 2009a; Evans et al. 2010]. The selection of the exposure control method should take into account the physical form of the material (e.g., bulk CNT and CNF, or materials containing CNT and CNF) and the task duration and frequency in which workers come into contact with the material. For instance, working with materials containing CNT or CNF (e.g., encapsulated in a solid) may require a different type of an exposure control system than would be required for large quantities of CNT and CNF in a highly dispersed free form; although, processes involving the cutting or grinding of solid materials containing CNT or CNF would require appropriate engineering controls (e.g., isolation/containment, local exhaust ventilation) to prevent aerosol release. Processes involved in the manufacturing (i.e., product collection at reactor), and the handling or transfer of dry bulk CNT or CNF should be performed in enclosed and ventilated systems. Whereas, local containment, such as low-flow vented work stations and small glove box chambers, can typically be applied to control exposure while handling research quantities of CNT and CNF in laboratories. All exhaust ventilation systems should be properly designed, tested, and routinely maintained [ACGIH 2007] to ensure maximum efficiency.

6.3 Work practices

Formal procedures (e.g., standard operating procedures [SOPs]) should be developed that include good work practices tailored to specific processes or work tasks and on the selection and use of personal protective equipment. All affected workers should be trained on these procedures. Management should

systemically review and update these procedures and convey to workers actions taken to resolve and/or improve workplace conditions.

An integral step to ensuring the health and safety of workers is the establishment of precautionary procedures and practices to minimize the risk of CNT and CNF exposure. Such procedures and practices should include:

- Educating workers on the safe handling (e.g., use of PPE) of CNT, CNF and CNT- and CNF- containing materials to minimize the likelihood of inhalation exposure and skin contact.

- Providing information, as needed, on the potential health risks associated with exposure to CNT and CNF with instructions on measures to prevent exposure.

- Providing facilities for hand-washing and encourage workers to make use of these facilities before eating, smoking, or leaving the worksite.

- Providing facilities for showering and changing clothes, with separate facilities for storage of non-work clothing, to prevent the inadvertent cross contamination of other areas (including take-home).

6.3.1 Clean-up and disposal

Procedures should be developed to protect workers from exposure to CNT and CNF during the clean-up of CNT and CNF spills and CNT- or CNF- contaminated surfaces. Inhalation and dermal exposures will likely present the greatest risks. The potential for inhalation exposure during clean-up will be influenced by the likelihood of CNT and CNF becoming airborne with bulk CNT and CNF (powder form) presenting a greater inhalation potential than CNT and CNF in solution (liquid form), and liquids in turn presenting a greater potential risk than CNT- and CNF-encapsulated materials.

It would be prudent to base strategies for dealing with spills and contaminated surfaces on the use of current good practices, together with available information

on exposure risks. Standard approaches for cleaning powder spills can be used for cleaning surfaces contaminated with CNT or CNF. These include using HEPA-filtered vacuum cleaners, wiping up CNT and CNF (powder form) using damp cloths, or wetting the powder prior to wiping. Liquid spills containing CNT or CNF can typically be cleaned by applying absorbent materials/liquid traps. If vacuum cleaning is employed, care should be taken that HEPA filters are installed properly and bags and filters changed according to manufacturer's recommendations. Dry sweeping or air hoses should not be used to clean work areas.

The handling and disposal of waste (including all cleaning materials) and other contaminated materials (e.g., gloves) should comply with all applicable regulations (e.g., federal, state, local).

6.4 Personal protective clothing

There are no regulations or guidelines for the selection of protective clothing or other apparel against exposure to CNT and CNF; however, the Occupational Safety and Health Administration (OSHA) require employers to provide employees with hand protection when exposed to hazards [OSHA 1910.138(a)]. Currently, limited information is available to accurately assess the exposure and health hazards of skin exposure to CNT and CNF. In a study to determine potential airborne and dermal exposures to SWCNT during manufacturing and handling, workers dermal exposure was estimated by placing cotton gloves over the rubber gloves used by workers [Maynard et al. 2004]. Dermal exposure estimates for SWCNT on individual gloves ranged from 217 µg to 6020 µg, with most of the SWCNT material appearing on the parts of the gloves in direct contact with surfaces. Results from experimental studies with other types of nanoparticles found that dermal penetration of nanoparticles may occur under certain conditions of exposure (e.g., flexing of skin) [Ryman-Rasmussen et al. 2006; Rouse et al. 2007]. Factors such as, size, shape, water solubility, and surface coating directly affect a nanoparticle's potential to penetrate the skin

[Sayes et al. 2004; Ryman-Rasmussen et al. 2006]. The results from *in vitro* studies, using primary or cultured human skin cells and engineered human skin, show that SWCNT and MWCNT are able to enter cells and cause the release of pro-inflammatory cytokines, induce free radical generation and oxidative stress, and decrease cell viability [Shvedova et al. 2003; Monteiro-Riviere et al. 2005; Murray et al. 2009]. Topical application of SWCNT (160 µg) to SKH-1 mice resulted in inflammation that was localized around or within the hair follicles; no significant changes were observed at the lowest dose (40 µg) tested [Murray et al. 2009]. However, the results of dermal toxicity testing with one type of MWCNT (Baytubes®) found no evidence of acute skin irritation or sensitization and only mild eye irritation in rabbits when tested according to OECD test guidelines [Pauluhn 2010b].

Given the limited amount of data on dermal exposure to CNT and CNF, it would be prudent to wear protective clothing and gloves when:

- all technical measures to eliminate or control release of exposure to CNT and CNF have not been successful or,
- in emergency situations.

If protective clothing and/or gloves are worn, particular attention should be given to preventing CNT and CNF exposure to abraded or lacerated skin. Based on limited experimental evidence, air-tight fabrics made of nonwoven textile seem to be more efficient in protecting workers against nanoparticles than fabrics made of woven cotton or polyester [Golanski et al. 2009; Golanski et al. 2010]. The challenge when selecting appropriate protective apparel is to strike a balance between comfort and protection. Garments that provide the highest level of protection (e.g., an impermeable Level A suit) are also the least comfortable to wear for long periods of time, while garments that are probably the least protective (e.g., thin cotton lab coat) are the most breathable and comfortable to wear. The efficiency of commercial gloves to prevent

dermal exposure to nanoparticles varies depending on the glove material, its thickness, and the manner in which it is used (e.g., stretched gloves, long exposure time) [NanoSafe 2008; Golanski et al. 2009; Golanski et al. 2010]. The proper selection of gloves should take into account the resistance of the glove to the chemical attack by both the nanomaterial and, if suspended in liquids, the liquid [USDOE 2007]. If protective gloves (e.g., nitrile, neoprene, latex) are used then "double gloving" may be needed when the worker requires physical protection (e.g., working with sharp instruments) in addition to chemical protection. Special attention should also be given to the proper removal and disposal of contaminated gloves to prevent skin contamination. Gloves should also be visually inspected for tears and routinely replaced.

6.5 Respirators

When engineering controls and work practices cannot reduce worker CNT and CNF exposures to below the REL then workers should be provided respiratory protection. In addition, the use of respirators may also be advisable for certain work tasks that place workers at risk of potentially high peak concentrations of CNT and CNF (e.g., the clean-up of CNT and CNF spills or debris, maintenance of equipment used to process CNT- and CNF-materials, the cleaning or disposal of filtration systems used to capture CNT and CNF aerosols). The OSHA respiratory protection standard (29 CFR 1910.134) sets out the elements for both voluntary and required respirator use. Elements of the standard include (1) a medical evaluation of the worker's ability to perform the work while wearing a respirator, (2) regular training of personnel, (3) periodic workplace exposure monitoring, (4) respirator fit-testing, and (5) respirator maintenance, inspection, cleaning, and storage. The program should be evaluated regularly and respirators should be selected by the person who is in charge of the program and knowledgeable about the workplace and the limitations associated with each type of respirator.

Based on published workplace monitoring data for CNT [Maynard et al. 2004; Han et al. 2008; Bello et al. 2008; Bello et al. 2009] and CNF [Methner et al. 2007; Evans et al. 2010], a NIOSH approved filtering-facepiece respirator or elastomeric half-facepiece particulate respirator equipped with a 95 or 100 series filter, should provide adequate protection when properly fit tested on the worker [Shaffer and Rengasamy [2009] and where engineered controls have been installed to reduce exposures. A properly fit-tested, half-facepiece particulate respirator or a filtering-facepiece respirator will provide protection at exposure concentrations up to 10 times the REL. Other classes of respirators exist that provide a higher level of protection (see Table 5). NIOSH provides guidance for selecting an appropriate respirator in the NIOSH Respirator Selection Logic 2004 [NIOSH 2005].

When selecting the appropriate respirator filter and determining filter change schedules, the respirator program manager should consider that particle overloading of the filters may occur because of the size and characteristics of CNT and CNF and the presence of other workplace aerosols. Based on this information, the respirator program manager may decide to choose a respirator with a higher assigned protection factor (APF) or choose a respirator with a higher level of laboratory filtration performance (e.g., changing from an N95 to a P100). Studies on the filtration performance of N-95 filtering-face piece respirators have found that the mean penetration levels for 40 nm particles range from 1.4% to 5.2%, indicating that 95 and higher performing respirator filters would be effective at capturing airborne CNT and CNF [Balazy et al. 2006; Rengasamy et al. 2007; Rengasamy et al. 2008].

6.6 Medical screening and surveillance

The evidence summarized in this document leads to the conclusion that workers occupationally exposed to CNT and CNF may be at risk of adverse respiratory effects. These workers may benefit from inclusion in a medical screening

program recommended as a prudent means to help protect their health (see Figure 1):

6.6.1 Worker participation

Workers who could receive the greatest benefit from medical screening include:

- Workers exposed to concentrations of CNT or CNF in excess of the REL (i.e., all workers exposed to airborne CNT or CNF at concentrations above 7µg/m^3 elemental carbon as an 8-hr TWA), or

- Workers in areas or in jobs, activities, or processes involving contact with CNT or CNF who have the potential for intermittent elevated airborne concentrations to CNT or CNF (i.e., workers involved in the transfer, weighing, blending, or mixing of bulk CNT or CNF, or the cutting and grinding of composite materials containing CNT or CNF, or workers in areas where such activities are carried out by others, are at risk of being exposed).

6.6.2 Program oversight

Oversight of the medical surveillance program should be assigned to a qualified health care professional who is informed and knowledgeable about potential workplace exposures, routes of exposure, and potential health effects related to CNT and CNF.

6.6.3 Screening elements

Initial evaluation

- An initial (baseline) evaluation should be conducted by a qualified health professional and should consist of:

- an occupational and medical history
- a physical examination with an emphasis on the respiratory system
- a spirometry test (Anyone administering spirometry testing as part of the medical screening program should have completed a NIOSH-approved training course in spirometry or other equivalent training.)
- a chest X-ray (All chest X-ray images should be interpreted by a NIOSH-certified B Reader using the standard International Classification of Radiographs of Pneumoconioses [ILO 2000 or the most recent equivalent].)
- other examinations or medical tests deemed appropriate by the responsible health care professional (The need for specific medical tests may be based on factors such as abnormal findings on initial examination.)

Periodic evaluations

- Periodic evaluations should be conducted at regular intervals (e.g., annual) or at other times (e.g., post-incident) as deemed appropriate for the individual worker by the responsible health care professional based on data gathered in the initial evaluation, ongoing work history, changes in symptoms such as new or worsening respiratory symptoms, and when process changes occur in the workplace (e.g., a change in how CNT or CNF are manufactured or used or an unintentional “spill’). Evaluations should include:
  - a respiratory symptom update
  - an occupational and medical history update
  - physical examination

- consideration of specific medical tests (e.g., spirometry, chest X-ray)

Written reports of medical findings

- The health care professional should give each worker a written report containing:
  - The individual worker's medical examination results.
  - Medical opinion(s) and/or recommendation(s) concerning any relationship(s) between the individual worker's medical condition(s) and occupational exposure(s), any special instructions on the individual's exposure(s) and/or use of personal protective equipment, and any further evaluation or treatment.
- For each examined employee, the health care professional should give the employer a written report specifying:
  - Any work or exposure restrictions based on the results of medical evaluations.
  - Any recommendations concerning use of personal protective equipment.
  - A medical opinion as to whether any of the worker's medical condition(s) is likely to have been caused or aggravated by occupational exposures.
- Findings from the medical evaluations having no bearing on the worker's ability to work with CNT and CNF should not be included in any reports to employers. Confidentiality of the worker's medical records should be enforced accordance with all applicable regulations and guidelines.

6.6.4 Worker training

Worker training should include information sufficient to allow workers to understand the nature of potential workplace exposures, routes of exposure, and instructions for reporting health symptoms. Workers should be provided with information about the purposes of medical screening, the health benefits of the program, and the procedures involved.

6.6.5 Periodic evaluation of data and screening program

- Standardized medical screening data should be periodically aggregated and evaluated to identify patterns of worker health that may be linked to work activities and practices that require additional primary prevention efforts. This analysis should be performed by a qualified health professional or other knowledgeable person to identify patterns of worker health that may be linked to work activities or exposures. Confidentiality of worker's medical records should be enforced in accordance with all applicable regulations and guidelines.
- Employers should periodically evaluate the elements of the medical screening program to ensure that the program is consistent with current knowledge related to exposures and health effects associated with occupational exposure to CNT and CNF.

Other important components related to occupational health surveillance programs, including medical surveillance and screening, are discussed in Appendix B.

7 Research Needs

Additional data and information are needed to assist NIOSH in evaluating the occupational safety and health concerns of working with CNT and CNF. Data are particularly needed on workplace exposures to CNT and CNF as well as information on whether in-place exposure control measures (e.g., engineering controls) and work practices are effective in reducing worker exposures. Additional assessment of NIOSH Method 5040 is needed to better understand potential interferences, improve the sensitivity and precision of the analytical method, and establish validity through the use of reference materials. The conduct of experimental animal studies with various types of CNT and CNF would help to elucidate potential mechanisms of toxicity and would provide a better understanding of the exposure parameters (e.g., mass, fiber number and size) that best describe the toxicological responses.

The following types of information and research are needed:

7.1 Workplace exposures, measurement, and controls

- Quantify worker airborne exposures to CNT and CNF.
- Evaluate NIOSH Method 5040 and other appropriate methods in CNT and CNF workplaces.
- Improve the sensitivity and precision of NIOSH Method 5040 and other appropriate methods for measuring airborne concentrations of CNT and CNF.
- Develop improved sampling and analytical methods for measuring airborne exposures to CNT and CNF that more closely align with the health endpoints and exposure metrics used in laboratory animal studies
- Determine the effectiveness of engineering controls to control airborne exposures to CNT and CNF below the NIOSH REL of 7 µg/m^3.

- Confirm the effectiveness of using HEPA filters in an exhaust ventilation system for removing exposures to CNT and CNF.
- Determine the effectiveness of gloves and other PPE barrier materials in preventing dermal exposure to CNT and CNF.
- Identify, quantify, and develop CNT and CNF reference materials for toxicology studies and for measurement quality control.

7.2 Experimental and human studies

- Conduct chronic animal inhalation studies to assess respiratory and other organ (e.g., heart and other circulatory system) effects. Special emphasis should be placed on assessing the risk for developing lung fibrosis and cancer. Studies should evaluate different types of CNT and CNF and use various exposure metrics (e.g., tube and fiber count, surface area) for assessing toxicological responses.
- Elucidate the mechanism(s) and other causative factors (e.g., tube and fiber size, surface area, and surface reactivity) by which CNT and CNF induce adverse effects (e.g., lung fibrosis) in animals.
- Develop early markers of exposure and pulmonary response to CNT and CNF given evidence from animal studies that CNT and CNF persist in the lungs and result in the development and progression of pulmonary fibrosis and/or cancer at relatively low mass doses.
- Quantitatively and qualitatively compare the CNT and CNF material used in the animal studies with the CNT and CNF materials found in workplace air.
- Determine the potential for CNT and CNF to penetrate the skin and cause toxicity.
- Evaluate the predictive value of using *in vitro* screening tests for assessing the hazard (e.g., fibrogenic potential) of various types of CNT and CNF.

- Assess the feasibility of establishing exposure registries for workers potentially exposed to CNT and CNF for the purpose of conducting future epidemiologic studies and surveillance activities.

Table 1. Findings from an uncharacterized carbon nanotube short-term intratracheal instillation toxicology study

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Huczko et al. 2001	G. pigs	ITI of soot containing CNT (uncharacterized)	25 mg [eval: 28 days post exposure]	NR	-	NR

NR: Not Reported

ITI: Intratracheal Instillation

+ : effect observed

- : no effect observed

Table 2a. Findings from published SWCNT short-term intratracheal instillation toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Warheit et al. 2004	Rats	ITI	1, 5 mg [eval: 24-h, 1-wk, 1 and 3 mo post exposure]	+ non-dose dependent	+ transient	-
Lam et al. 2004	Mice	ITI	0.1, 0.5 mg [eval: 7 or 90 days post exposure]	+	+	NR
Inoue et al. 2008	Mice	ITI	4 mg [eval: 24-h post exposure]	NR	+	NR

NR: Not Reported

ITI: intratracheal instillation

+ : effect observed

- : no effect observed

Table 2b. Findings from published SWCNT short-term aspiration toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Shvedova et al. 2005	Mice	Pharyngeal aspiration	10, 20, 40 µg [eval: 1, 3, 7, 28 and 60 days post exposure]	+	+	+
Mangum et al. 2006	Rats	Pharyngeal aspiration	2 mg/kg [eval: 1 or 21 days post exposure]	+	-	+ (interstitial lesions)
Shvedova et al. 2007	Mice (vitamin E deficient)	Pharyngeal aspiration	40 µg [eval: 1, 7, and 28 days post exposure]	+	+	+
Mercer et al. 2008	Mice	Pharyngeal aspiration	10 µg [eval: 1-h, 1 and 7 days and 1 mo post exposure]	+(undispersed) - (dispersed)	+	+
Shvedova et al. 2008	Mice	Pharyngeal aspiration	5,10, 20 µg [eval: 1, 7, and 28 days post exposure]	+	+	+

NR: Not Reported

+: effect observed

-: no effect observed

Table 2c. Findings from published SWCNT short-term inhalation toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Shvedova et al. 2008	Mice	Inhalation	5mg/m^3 5h/day for 4 days [eval: 1, 7 and 28 days post exposure]	+	+	+

NR: Not Reported

+: effect observed

-: no effect observed

Table 3a. Findings from published MWCNT short-term intratracheal instillation toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Muller et al. 2005	Rats	ITI	0.5, 2, 5 mg [eval: 1-h, 3, 15, 28 and 60 days post exposure]	+	+	+
Huczko et al. 2005	G. pigs	ITI	15 mg [eval: 90 days post exposure]	NT (pneumonia-like reaction)	+(Increased lung resistance)	+/-
Grubek-Jaworska et al. 2005	G. Pigs	ITI	12.5 mg [eval: 90 days post exposure]	+	+	+
Carrero-Sanchez et al. 2006	Mice	ITI	1, 2.5, 5 mg/kg [eval: 1, 2, 3, 7 and 30 days post exposure]	+	+	+
Deng et al. 2007	Mice	ITI	600 µg [eval: 1 day post exposure]	NR	-	NR
Li et al. 2007	Mice	ITI	0.05 mg [eval: 8, 16, and 24 days post exposure]	NR	+	NR
Liu et al. 2008	Rats	ITI	1,3,5,7 mg/kg [eval: 1and 7 days, 1 and 3-mo post exposure]	+	+	NR
Muller et al. 2008a	Rats	ITI	2 mg [eval : 3 and 60 days post exposure]	+	+	NR
Muller et al. 2008b	Rats	ITI	0.5 or 2 mg [eval : 3 days post exposure]	NR	+	NR
Inoue et al. 2008	Mice	ITI	4 mg/kg [eval: 1 day post exposure]	NR	+	NR
Elgrabli et al. 2008a	Rats	ITI	1, 10, 100 µg [eval: 1, 7, 30, 90 and 180 days post exposure]	-	-	-

NR: Not Reported

+: effect observed

-: no effect observed

Table 3b. Findings from published MWCNT or CNF short-term aspiration toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Sriram et al. 2007	Mice	Pharyngeal aspiration	MWCNT 10, 20, or 40 µg	+	+ Including neuroinflammation of the brain	NR
Han et al. 2008	Mice	Pharyngeal aspiration with ozone exposure	MWCNT 20 µg [eval: 5 and 24-h post exposure]	NR	+	NR
Hubbs et al. 2009	Mice	Pharyngeal aspiration	MWCNT-20 or 80 µg [eval: 7 and 56 days post exposure]	+	+	+
Sriram et a. 2009	Mice	Pharyngeal aspiration	MWCNT-10 or 80 µg [eval: 1, 7, 28 days post exposure]	NR	neuroinflammation	NR
Wolfarth et al. 2009	Mice	Pharyngeal aspiration	MWCNT- 40 µg [eval: 1, 7, 28, 56 days post exposure]	+	+	+
Porter et al. 2010	Mice	Pharyngeal aspiration	MWCNT-10,20,40, or 80 µg [eval: 1, 7, 28 days post exposure]	+	+	+
Han et al. 2010	Mice	Pharyngeal aspiration	MWCNT-20 or 40 µg [eval: 1 and 7 days post exposure]	NR	+	NR
Kisin et al. 2010	Mice	Pharyngeal aspiration	CNF- 40 or 120 µg [eval: 1, 7, and 28 days and 1 year post exposure]	+	+	+

NR: Not Reported

+: effect observed

-: no effect observed

Table 3c. Findings from published MWCNT short-term inhalation toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Li et al. 2007	Mice	Inhalation	Est. lung deposition dose : 0.07, 0.14, .21mg. [eval : at days 8, 16 and 24]	NR	-	NR
Mitchell et al. 2007	Mice	Inhalation	0.3, 1, 5 mg/m^3 6h/day for 7 or 14 days. [eval: at days 7 and 14]	-	-	-
Arkema 2008	Rats	Head-nose inhalation	0.1, 0.5, 2.5 mg/m^3 6h/day for 5-days. [eval: at days 7 and 28]	- (0.1 mg/m^3) + (0.5, 2.5 mg/m^3)	- (0.1 mg/m^3) + (0.5, 2.5 mg/m^3)	-
Ryman-Rasmussen et al. 2009a	Mice w/preexisting allergic inflammation	Nose-only inhalation	100 mg/m^3 for 6hr (~10mg/kg alveolar dose). [eval: at days 1 and 14]	Lung injury	+	+ when preexisting allergic inflammation exists
Ma-Hock et al. 2009[1]	Rats	Head-nose inhalation	0.1, 0.5, 2.5 mg/m^3 6h/day-5days/wk for 13-weeks. [eval: at week 13]	+	+	-
Porter et al. 2009	Mice	Whole body inhalation	10 mg/m^3 5h/day for 2, 4 and 8 days then evaluated	+	+	+
Sriram et al. 2009	Mice	Whole body inhalation	10 mg/m^3 5h/day for 2, 4 and 8 days then evaluated	NR	Neuro-inflammation	NR

Table 3c. (Continued) Findings from published MWCNT short-term inhalation toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Ellinger-Ziegelbauer 2009	Rats	Nose-only inhalation	11 and 241 mg/m^3 for 6hr. [eval: at days 7, 28, and 90]	NR	+	NR
Ryman-Rasmussen et al. 2009b	Mice	Nose-only inhalation	1 or 30 mg/m^3 for 6hr. (~0.2mg/kg and 4mg/kg alveolar dose). [eval: at 1 day, and 2, 6, and 14 weeks]	-	- (1 mg/m^3) + (30 mg/m^3)	- (1 mg/m^3) + (30 mg/m^3)

Table 3d. Findings from published MWCNT or SWCNT short-term injection/implantation toxicology studies

Study Design and Exposure/Dose				Observed Pulmonary Effects		
Author/Year	**Species**	**Exposure Route**	**Exposure or Dose**	**Granuloma**	**Inflammation**	**Fibrosis**
Deng et al. 2007	Mice	Intravenous injection (also gavage)	1 - 600 µg MWCNT depending on exp. route	NR	-	NR
Takagi et al. 2008	Mice	Intraperitoneal injection	27.5 % longer than 5 µm; 1 x 10^9 MWCNT/1 mL (corresponds to 3 mg) [eval: week 25	Mesothelioma	+	+
Poland et al. 2008	Mice	Intraperitoneal injection	Long and short MWCNT 50 µg [eval: 1 and 7 days post exposure]	Increase in response with increasing fiber length	Increase in response with increasing fiber length	NR
Muller et al. 2009	rats	Intraperitoneal injection	MWCNT < 1 µm on avg. length; 2 or 20 mg w/defects, 20 mg wo/defects. [eval: month 24]	No mesotheliomas		
Sakamoto et al. 2009	rats	Intrascrotal injection	MWCNT > 5 µm in length; 0.24 mg (1 mg/kg body weight) 27.5 %	Mesotheliomas	NR	NR
Varga and Szendi 2010	rats	Peritoneal implantation	10 mg of MWCNT or SWCNT (contained in a gelatin capsule)	No mesotheliomas	-	-

NR: Not Reported

+: effect observed

-: no effect observed

Table 4. Recommended Occupational Exposure Limits for CNT

Reference	Occupational Exposure Limit (OEL)	Comments
Pauluhn J [2010b]	0.05 mg/m^3 TWA for MWCNT	Prevent lung clearance overload and pulmonary inflammation for MWCNT (Baytubes®). Based on rat subchronic inhalation study.
Kobayashi et al. 2009	0.21 mg/m^3 TWA (8h/day-5day/wk) for MWCNT*	Prevent pulmonary inflammation for one type of MWCNT. Based on rat subchronic (4-wk) inhalation study at 0.37 mg/m^3 (NOAEL). Human lung deposition of MWCNT calculated from rat data and an uncertainty factor of 2 applied to derive OEL
BSI 2007	0.01 fibers/ml for fibrous nanomaterials with high aspect ratios (<3:1 and length >5000nm)	Benchmark exposure level (BEL) based on one tenth of the asbestos exposure limit

Note: * NIOSH recalculated the OEL reported by Kobayashi et al. 2009 and derived an OEL of 21 µg/m^3 (0.021 mg/m^3). [see Chapter 5]

Table 5. Respiratory protection for exposure to CNT and CNF

Airborne concentrations of CNT and CNF or conditions of use[1] options	Minimum respiratory protection
7-70 µg/m^3 (10 x REL)	Any filtering-facepiece respirator or air-purifying, elastomeric half-facepiece respirator equipped with appropriate type of particulate filter[2] Any negative pressure (demand), supplied –air respirator equipped with a half-mask
≤175 µg/m^3 (25 x REL)	Any powered, air purifying respirator equipped with a hood or helmet and a high-efficiency particulate air filter (HEPA filter) Any continuous flow supplied-air respirator equipped with a hood or helmet
≤350 µg/m^3 (50 x REL)	Any air-purifying full-facepiece respirator equipped with N-100, R-100, or P-100 filter Any powered air-purifying respirator equipped with a tight-fitting half-facepiece and a high-efficiency particulate air filter. Any negative pressure (demand) supplied-air respirator equipped with a full-facepiece Any continuous flow supplied-air respirator with a tight-fitting half-facepiece Any negative pressure (demand) self-contained respirator equipped with a full-facepiece

Table 5. (Continued) Respiratory protection for exposure to CNT and CNF

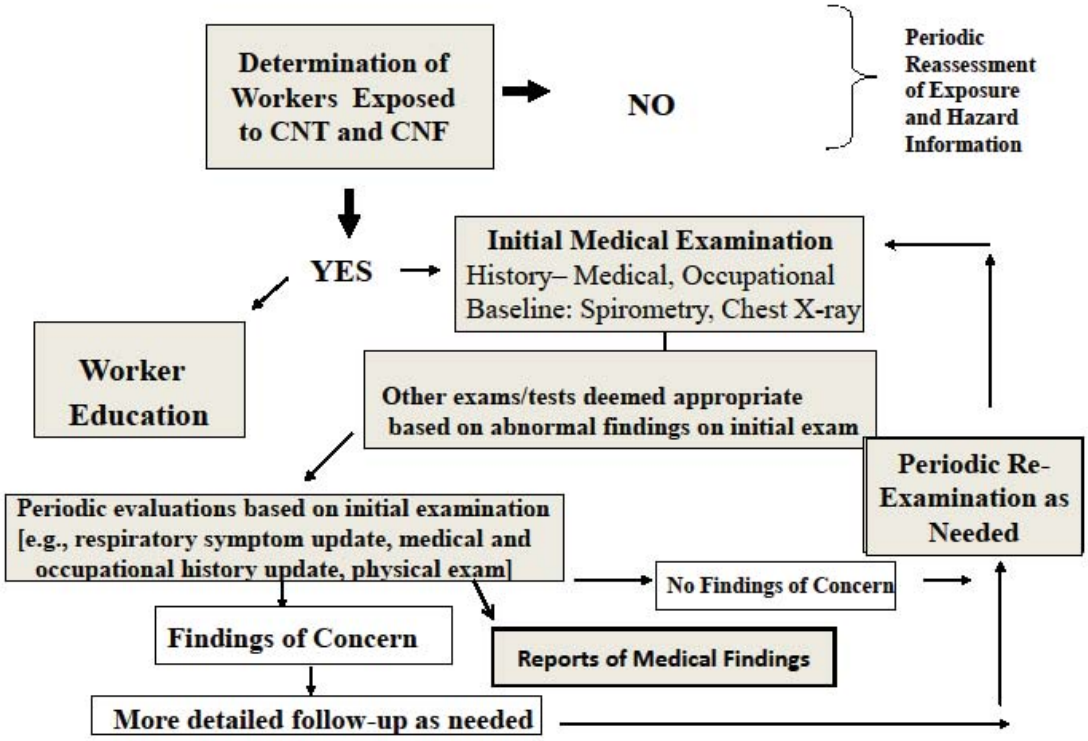
Airborne concentrations of CNT and CNF or conditions of use[1] options	Minimum respiratory protection
≤7000 µg/m^3 (1,000 x REL)	Any pressure-demand supplied-air respirator equipped with a full-facepiece

1 The protection offered by a given respirator is contingent upon (1) the respirator user adhering to complete program requirements (such as the ones required by OSHA in 29 CFR 1910.134), (2) the use of NIOSH-certified respirators in their approved configuration, and (3) individual fit testing to rule out those respirators that cannot achieve a good fit on individual workers.

2 The appropriate type of particulate filter means: Any 95 or 100 series (N, R, or P) filter. Note: N-95 or N-100 series filters should not be used in environments where there is potential for exposure to oil mists.

Note: complete information on the selection of respirators can be found at: (1) OSHA report-3352-02 2009 Assigned Protection Factors for the Revised Respiratory Protection Standard at: http://www.osha.gov/Publications/3352-APF-respirators.html and (2) NIOSH at: http://www.cdc.gov/niosh/docs/2005-100/default.html

Figure 1. Medical Surveillance Recommendations – CNT and CNF



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APPENDIX A

QUANTITATIVE RISK ASSESSMENT OF CNT

A.1 Introduction

The increasing production and use of CNT and the preliminary significant toxicology findings necessitates an assessment of the potential adverse health effects in workers who produce or use these materials. Risk assessment provides a tool to characterize the health risk of exposure to a hazard, as well as to examine the uncertainties and additional research needs. The classical paradigm for risk assessment in the U.S. includes four steps: hazard assessment, exposure assessment, dose-response analysis, and risk characterization [NRC 1983; 2009]. Research studies in toxicology, epidemiology, exposure measurement, and other areas provide the data needed for these risk assessment steps. Animal studies of CNT that have been published to date include several subchronic and short-term studies, which provide information on the dose-response relationship and the biological mechanisms of early-stage, adverse lung effects. No chronic animal studies of CNT were available. No epidemiological studies of workers producing or using CNT were available.

Risk assessment practice seeks to use the best available scientific methods and evidence as the basis for public and occupational health decision making [NRC 2009]. When sufficient dose-response data are available (e.g., from animal studies), quantitative risk assessment can be performed. Quantitative risk assessment provides estimates of the severity and likelihood of an adverse response associated with exposure to a hazardous agent [Piegorsch and Bailer 2005; NRC 2009]. Quantitative risk estimates provide the health basis for developing occupational exposure limits, evaluating the effectiveness of current industrial hygiene practices and exposure controls, improving sampling and analytical methods, and developing other risk management strategies needed to protect workers' health.

A.2 Methods for NIOSH Risk Assessment

NIOSH used benchmark dose (BMD) modeling [Crump 1984; 1995; EPA 2010] of rodent dose-response data to estimate risk and working lifetime exposure concentrations as a basis for developing a NIOSH recommended exposure limit (REL) for CNT. Dose-response data from subchronic and short-term studies in rats and mice exposed to SWCNT or MWCNT were used to estimate the BMDs associated with benchmark responses (BMRs) of early-stage, adverse lung responses. The rodent-based BMD estimates were extrapolated to humans by accounting for species differences in factors influencing lung dose to estimate the working lifetime risk of airborne exposure to CNT.

When feasible, NIOSH utilizes BMD estimates in risk assessment rather than a lowest observed adverse effect level (LOAEL) or a no observed adverse effect level (NOAEL) for the following reasons: (1) BMD methods provide a standardized method for risk estimation (2) BMD methods provide both maximum likelihood (BMD) and 95% lower confidence limit (BMDL) estimates, which take into account the sample size and variability in the data; and (3) BMD models use all (or most) of the dose-response data in estimating the BMD(L)s. In contrast, with NOAELs and LOAELs: (1) values can vary depending on the dose group spacing; (2) NOAELs and LOAELs are not risk-based, and do not statistically account for the number of observations or variability in the data; and (3) NOAELs and LOAELs are based on only one dose group. Yet, BMD estimation requires more dose-response data than does a NOAEL or LOAEL. Sparse data especially near the BMR provides limited information for BMD estimation and can result in model uncertainty. Comparison of the BMD(L) estimates to the LOAELs or NOAELs provides a check on the estimated and observed responses in the low dose region of the data.

A.2.1 Rodent dose-response data

All of the published rodent studies on pulmonary responses to CNT (Section 3, Tables 1-3c) were examined for possible inclusion in this risk assessment. Pulmonary effects were examined because of their relevance to workers who may be exposed to CNT in workplace air. The studies with adequate quantitative dose-response data to estimate BMDs were included in these analyses. These studies reported the route of exposure, doses, duration of exposure or post-exposure, the number of animals per group, and lung responses, as well as information on the size and characterization of the CNT (Table A-1). In general, the CNT animal studies have limited data, with few (4-20) animals per dose group and sparse dose group spacing especially in the low range of the dose-response curve. Some of these studies just meet the minimum data criteria for BMD estimation, i.e., a graded monotonic response with dose and at least two dose groups in addition to the unexposed (control) group [EPA 2000]. Although it is preferable to have one or more doses near the benchmark response (e.g., 10%) [EPA 2000], in some studies the response proportions were quite high at each dose (e.g., 30-100%) [Lam et al. 2004; Ma-Hock et al. 2009; Pauluhn 2010a]. In addition, one study [Shvedova et al. 2008] had only one dose group in addition to the control, but was included because it is the only animal inhalation study for SWCNT currently available and provides useful comparison by route of exposure.

Either individual animal response data or the mean and standard deviation of the group response were required for BMD model fitting. The dose was either the intratracheal instillation (IT) or pharyngeal aspiration (PA)-administered mass dose (mg/lung) or the inhaled mass concentration (mg/m^3). Datasets with treatment-related mortality of animals were not used. Data on special preparations of CNT (e.g., ground CNT) or studies using sensitive animal models (e.g., vitamin E deficient) were not included (although these data may be of interest for subsequent analyses using animal models to investigate biological mechanisms including in sensitive human populations, or to evaluate the effect of altering CNT properties on hazard potential).

Study details of the data selected for this risk assessment are provided in Table A-1. These studies include the two recently published subchronic inhalation studies of MWCNT in rats [Ma-Hock et al. 2009; Pauluhn 2010a]; and several IT, PA, or short-term inhalation studies in rats or mice exposed to SWCNT [Lam et al. 2004; Shvedova et al. 2005, 2008] or MWCNT [Muller et al. 2005; Ellinger-Ziegelbauer and Pauluhn 2009; Porter et al. 2010] with post-exposure durations and examination at 32 to 91 days after exposure. NIOSH considers the subchronic inhalation studies to be the best available data for extrapolation to human occupational exposure conditions.

In the subchronic inhalation studies, rats were head-nose exposed [Ma-Hock et al. 2009] or nose-only exposed [Pauluhn 2010a] to three or four different airborne mass concentrations (6 hr/d, 5 d/week) for 13-weeks. Lung responses were examined at the end of the 13-week exposure in both studies; post-exposure follow-up was extended to 6 months in the Pauluhn [2010a] study. A study of 1-day inhalation exposure to MWCNT (Baytubes) in rats and examined 13 weeks after the end of exposure [Ellinger-Ziegelbauer and Pauluhn 2009] provided an opportunity to compare the dose-response relationships of the 1-day inhalation exposure study with that of the 13-week (subchronic) inhalation study [Pauluhn 2010a] to examine the influence of dose-rate on the rat lung responses. These findings are relevant to interpreting and using the results from the short-term exposure studies of the SWCNT and other MWCNT.

The IT, PA, and short-term inhalation studies provide additional data to compare the dose and lung responses to other MWCNTs or SWCNTs, with different types and amounts of metal contaminants. Although both IT and PA routes bypass the head region and deliver the CNT material directly to the trachea and lung airways, PA is considered more similar to inhalation than IT because PA provides greater dispersion of deposited material in the lungs [Shvedova et al. 2005; 2008]. Following the administered dose (on day 1), the lung responses were evaluated after a post-exposure period (e.g., 1, 7, 28, 60, and/or 90 days). For studies, with more than one post-exposure duration, the longest post-exposure duration data are used in these risk analyses. Some of these studies also provide data on other particles or fibers (e.g.,

ultrafine carbon black, crystalline silica and asbestos) for comparison of dose and response to that from MWCNT or SWCNT [Lam et al. 2004; Muller et al. 2005; Shvedova et al. 2005].

The lung response measures used in this risk assessment are either dichotomous (proportion of animals observed with the response endpoint) or continuous (amount or level of response in individual animals) (Table A-1). The dichotomous responses include the incidence of lung granulomas [Lam et al. 2004]; granulomatous inflammation [Ma-Hock et al. 2009], and focal interstitial (septal) thickening [Ellinger-Ziegelbauer and Pauluhn 2009; Pauluhn 2010a]. The continuous responses include the amount of hydroxyproline (as mass) [Muller et al. 2005] and alveolar epithelial connective tissue thickness [Shvedova et al. 2005, 2008]. In addition, one study reported an ordinal response measure (i.e., fibrosis severity score) [Porter et al. 2010]. Because BMD methods for ordinal data are not readily available, this ordinal response was converted to a dichotomous response for this analysis (as described in section A.2.3).

Collectively, the data available for CNT risk assessment include dose-response data from several rodent species/strains, males and females, and three routes of exposure to several types of SWCNT and MWCNT with varying types and amounts of metal contaminants (Table A-1). The dose metric used in this risk assessment is the mass dose of CNT in the lungs, either the administered dose (IT or PA studies) or the lung burden (deposited or retained) estimated from the airborne exposure concentration (inhalation studies). Mass dose was used because all of the studies reported this dose metric.

A.2.2 Estimated Lung Dose in Animals

For the IT and PA studies [Lam et al. 2004; Shvedova et al. 2005; Muller et al. 2005; Porter et al. 2010], the administered CNT mass dose was assumed to be equivalent to the deposited lung dose. In the inhalation studies [Shvedova et al. 2008; Ma-Hock et al. 2009; Ellinger-Ziegelbauer and Pauluhn 2009; Pauluhn 2010a], the deposited lung dose

was estimated from the exposure concentration and duration, the species-specific ventilation rate, and the alveolar deposition fraction (estimated from the CNT aerodynamic particle size data), as follows:

Deposited lung dose (μg) = (A.1)

Exposure Concentration (μg/m^3) x Duration (hr/d x d/wk x wk)
x Minute Ventilation (L/min) x 0.001 m^3/L x 60 min/hr
x Alveolar Deposition Fraction

The exposure concentration and duration, as reported in the studies, are shown in Table A-1. The values used for respiratory minute ventilation were based on the species and body weight: 0.037 L/min for mice [EPA 1988; 2006]; 0.25 L/min for male rats in Pauluhn [2010] (369g body weight); and 0.21 L/min for male and female rats in Ma-Hock et al. [2009] assuming average body weight of 300g EPA [1995; 2006]). The alveolar lung deposition fraction in rats was estimated from the MPPD 2.0 model for inhaled poorly-soluble spherical particles [CIIT and RIVM 2006] using the mass-median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) data reported for SWCNT and MWCNT (Table A-2). In the mouse inhalation study [Shvedova et al. 2008], an alveolar deposition fraction of 0.01 was estimated based on the MMAD (Table A-2) and interpolating from the deposition fractions for monodisperse spherical particles reported in Table 2 of Raabe et al. [1988].

For the two subchronic inhalation studies of MWCNT [Ma-Hock et al. 2009; Pauluhn 2010a], the retained lung dose in rats was also estimated. The MPPD 2.0 model [CIIT and RIVM 2006] was used to estimate the lung burden at the end of the 13-week exposure based on the particle MMAD and GSD (Table A-2) reported in those studies, assuming unit density (the lowest density accepted by MPPD 2.0). Ma-Hock et al. [2009] reported the MWCNT particle density of 0.043 g/ml, and Pauluhn [2010a] reported the MWCNT particle density of 0.1-0.3 g/ml. In the absence of CNT-specific lung models, the deposition and clearance of CNT in the lungs was assumed to be

equivalent to that of spherical particles with equivalent aerodynamic size (MMAD and GSD).

A.2.3 Animal dose-response modeling and BMD estimation

The dose-response data in rats and mice exposed to SWCNT or MWCNT were modeled using benchmark dose methods [Crump 1984; EPA 2010]. A benchmark dose has been defined as ". . . a statistical lower confidence limit for the dose corresponding to a specified increase in level of [adverse] health effect over the background level" [Crump 1984]. The increased level of adverse effect (called a benchmark response or BMR) associated with a BMD is typically in the low region of the dose-response data (e.g., a 10% excess risk). In this document, the term BMD is used to describe the point estimate based on maximum likelihood estimation, and the term BMDL is used to describe the lower 95% confidence limit (i.e., as originally defined by Crump [1984]). A 10% excess risk based on dichotomous or quantal data is used because a 10% response is at or near the limit of sensitivity in the animal bioassay [EPA 2000]. The BMD(L) associated with a 10% BMR is used as a point of departure (POD) for low dose extrapolation using linear or nonlinear methods (depending on the mode of action evidence). The low dose extrapolation may include estimation of the probability of effects at low doses or a reference value (not risk-based) by accounting for uncertainties in the dose estimation (e.g., extrapolation form animal to human, inter-individual variability, limitations in the animal data [EPA 2000].

A.2.3.1 Dichotomous response data

For dichotomous data (yes/no response), a BMD is defined as the dose associated with a specified increase in the probability of a given response, either as an excess risk (i.e., additional probability above background) or as a relative risk (i.e., relative to the background probability of having a normal response) [Crump 2002].

In this analysis, the BMD (using dichotomous data) is the dose *d* corresponding to a specified excess (added) risk (e.g., 10%) in the proportion of animals with a given adverse lung response (BMR), or:

$$BMR = P(d) - P(0)$$

where *P(d)* is the probability of an adverse response at the BMD, and *P(0)* is the probability of that adverse response in an unexposed population [Crump 2002; EPA 2010].

The dichotomous BMR lung responses include the presence or absence of granulomatous inflammation [Ma-Hock et al. 2009] or focal septal thickening [Pauluhn 2010a] (Table A-1). The proportion of animals responding with the minimal or higher severity was selected as the benchmark response. The BMD(L) estimates are expressed as the mass dose of SWCNT or MWCNT in rodent lungs associated with the specified BMR. These animal-based BMD(L)s are extrapolated to humans based on species-specific differences in the estimated deposition and retention of CNTs in the lungs (Section A.2.4).

In Porter et al. [2010], the fibrosis pathology score is an ordinal measure of the fibrotic response, based on the sum of the scores for the severity (none=0, minimal=1, mild=2, moderate=3, marked=4, severe=5) and distribution (none=0, focal=1, locally extensive=2, multifocal=3, multifocal and coalescent=4, diffuse=5) of the fibrotic lesions in the lungs [Hubbs et al. 1997]. Because there is no readily available BMD software for ordinal data currently, the ordinal score for fibrosis severity in Porter et al. [2010] was converted to a dichotomous measure of having attained a fibrosis severity score of 4. Because a response of fibrosis severity score <4 was 100% in all exposed groups, a BMD could not be estimated for those BMRs. The lowest quantifiable BMR was a fibrosis pathology score of 4 or greater, which could arise from histopathology scores of either minimal (1), multi-focal (3) lesions; moderate (3), focal (1) lesions; or mild (2), locally-extensive (2) lesions. As with the other early lung responses observed in these

short-term or subchronic CNT studies, the biological functional significance of a fibrosis severity score of 4 is not known, although this response was highly statistically significantly elevated compared to controls and persisted with increasing post-exposure time from 28 to 56 days.

A.2.3.2 Continuous response data

BMD estimation using continuous data requires specifying a BMR level along the continuum of responses. Continuous response data provide information on the amount or degree of a biological response. Continuous response measures may include nonzero, normal levels that are associated with the normal structure or function (e.g., a certain number immune cells or amount of protein in healthy lungs). These levels can become elevated in response to a toxicant, and at some point may result in irreversible, functional impairment of the lungs [NIOSH 1986]. If data are available, the BMRs can be based on a biologically-significant response that is associated with, or expected to result in, a material impairment of health. However, there may be insufficient data to determine a specific level that is associated with a measurable adverse response. In that case, a statistical criterion may be used as a BMR for continuous data.

A statistical method (originally referred to as a "hybrid" method) is described by Crump [1995] to provide BMD(L) estimates from continuous data that are equivalent to a 10% excess risk based on dichotomous data, assuming an abnormal or biologically significant response is defined as the upper 99^{th} percentile of the control distribution. In this method, "…setting BMR = 0.1 and P_O = 0.01 is equivalent to choosing the BMD to be the dose that results in an increase in the mean equal to 1.1 times the standard deviation," assuming a Normal distribution with constant variance [Crump 1995]. That is, if one assumes that the probability of the specified adverse response in the unexposed population is the upper 1% of a Normal distribution of responses, then selecting a BMR of 1.1 standard deviations above the control mean response is equivalent to a 10% BMD as estimated in dichotomous data.

In this analysis, previous animal studies using the pulmonary fibrosis measure of alveolar connective tissue thickening and pulmonary function (associated with chronic ozone exposure) in rats [Chang et al. 1992; Costa et al. 1995; Stockstill et al. 1995] were evaluated as a possible basis for a BMR using this same pulmonary fibrosis response in the CNT animal studies. However, those findings did not appear to extrapolate well to the mice in Shvedova et al. [2005, 2008]. That is, the observed abnormal response in rats (associated with a persistent lung function deficit) was a 36% increase in the control mean alveolar connective tissue thickness [Chang et al. 1992; Costa et al. 1995]; however this amount of response occurred in up to 30% of the control (unexposed) mice in Shvedova et al. [2005, 2008] (vs. in 2.5% of controls in Chang et al. 1992), in part due to the greater variability in the alveolar tissue thickness in the unexposed mice. In addition, no data were found of a biologically-relevant BMR for the amount of hydroxyproline in the lungs of rats or mice. Developing criteria for biologically-based BMRs is an area for future research with regard to interpreting and using animal dose-response data for risk assessment in humans. In the absence of a biological basis for a BMR for the continuous response measures of alveolar connective tissue thickening or the amount of hydroxyproline, NIOSH used the statistical criterion described by Crump [1995], in which a BMR of 1.1 standard deviations above the control mean response is equivalent to a 10% excess risk in the dichotomous data, assuming the 99th percentile of the distribution of control responses is abnormal or biologically significant.

That is, the BMR used for the continuous data is defined as

$$BMR = \mu(d) - \mu(0)$$

where $\mu(d)$ is the mean response at the BMD (d); $\mu(0)$ is the control mean response; and BMR is the specified number of standard deviations $(StDev)$ (i.e., 1.1 in these analyses). That is, the continuous data-based BMD is the dose associated with a 10% increase in the proportion of animals exposed at dose d with response greater than the 99th percentile of the control mean response.

The estimates of $\mu(d)$ and $\mu(0)$ *are* derived from the fitted dose-response models (polynomial) (Section A.2.3.3). In this analysis, the BMR lung response is based on continuous data including alveolar connective tissue thickness and hydroxyproline amount (Table A-1).

A.2.3.3 BMD model fitting

The animal dose-response data were fit using the benchmark modeling software (BMDS 2.1.2) [EPA 2010]. The dichotomous data were fit with a multistage (polynomial degree 2) model. This model was selected because it was the only model that provided adequate fit to the subchronic inhalation data, each of which [Ma-Hock et al. 2009; Pauluhn 2010] had only one dose between zero and 100% response. The other BMDS models failed to converge or, in further statistical evaluation, showed non-unique parameter solutions. The continuous dose-response data were fit with a polynomial model of degree 2 for all data with three or more dose groups, and degree 1 (linear) for data with two groups.

P-values for goodness of fit were computed for the individual BMDS models (based on likelihood methods) [EPA 2007]. Model fit was considered adequate at $p>0.05$ (i.e., testing for lack of fit), although the p-values based on likelihood ratio tests may not be a reliable indicator of model fit in the studies with few animals per group. The number of animals per dose group in each study is given in Table A-1. EPA typically uses a $p>0.1$ criteria for BMD model fit [EPA 2000]. Either criteria are considered reasonable and represent a trade-off in the type I or type II error. That is, $p>0.1$ provides more power to reject an incorrect model, while $p>0.05$ provides less chance of rejecting a correct model. The BMD model fits to each data set are shown in Figures A-1 (subchronic studies), A-2 (short-term studies, dichotomous response), and A-3 (short-term studies, continuous response).

A.2.4 Human-equivalent dose and working lifetime exposure

The rodent BMD(L)s were extrapolated to humans based on species-specific differences in the alveolar epithelial surface area of the lungs. It is assumed that humans and animals would have equal response to an equivalent dose (i.e., mass of CNT per unit surface area of lungs). The human-equivalent BMD(L)s were the target lung doses used to estimate, respectively, the maximum likelihood estimate (MLE) and 95% lower confidence limit (95% LCL) of the 8-hr time-weighted average (TWA) exposure concentration during a 45-year working lifetime.

The human-equivalent BMD(L) was calculated as follows:

$$\text{Human-equivalent BMD(L)} = \text{Rodent BMD(L)} \times [\text{Alv SA human} / \text{Alv SA rodent}] \quad \text{(A.2)}$$

where the values used for alveolar lung surface area (Alv SA) were 102 m^2 (human) [Stone et al. 1992]; 0.4 m^2 (rat) and 0.055 m^2 (mouse) [Mercer et al. 2008]. In Tables A-3 – A-5, the human-equivalent BMD(L)s were multiplied by 0.001 mg/µg to obtain the units of mg per lung.

The human-equivalent BMD(L)s are expressed as the mass (mg) of CNT in the lungs. The working lifetime airborne mass concentration that would result in the BMD(L) human-equivalent lung mass dose was calculated based on either deposition only (no lung clearance) or retention (lung deposition and clearance), as described below.

(a) Deposited lung dose

$$\text{Estimated 8-hr TWA } (\mu g/m^3) = \text{Human-equivalent BMD(L) } (\mu g) \times / \quad \text{(A.3)}$$

[8-hr worker air inhaled (m^3/day) x Alveolar Deposition Fraction x Work Days]

The values assumed include: 9.6 m^3 8-hr air intake (reference worker [ICRP 1994]); alveolar deposition fraction based on aerodynamic particle size (Table A-2); and working lifetime days (250 days/yr x 45 yr).

(b) Retained lung dose

The MPPD 2.0 human model [CIIT and RIVM 2006] for inhaled poorly-soluble spherical particles was used to estimate the working lifetime exposure concentration that would result in the human-equivalent BMD(L) lung burden. This was accomplished by a systematic search to identify the 8-hr time weighted average (TWA) airborne concentration over a 45-year working lifetime that predicted the target lung burden. The input parameters used in the MPPD human model (Yeh and Schum model option) include: CNT aerodynamic particle size (MMAD, GSD) (Table A-2); inhalability adjustment; oronasal-normal augmenter; and reference worker conditions, including 9.6 m^3 of air inhaled per 8-hr day (corresponding to 17.5 breaths/min and tidal volume of 1143 ml), and work for 8-hr/d, 5 d/wk, 50 wk/yr, and 45-years.

In the two subchronic inhalation studies for MWCNT, excess risk estimates were derived based on either the estimated deposited lung dose or the estimated retained lung dose [Ma-Hock et al. 2009; Pauluhn 2010a].

A.3 Results

A.3.1 Benchmark dose and working lifetime exposure estimates

The estimates of the rodent BMD(L)s, the human-equivalent BMD(L)s, and the associated working lifetime 8-hr TWA exposure concentrations (MLE and 95% LCL), called the benchmark concentration (BMC) and the BMCL (95% LCL of the BMC), are shown in Tables A-3 through A-5. All dose-response models used in this risk

assessment provided adequate fit ($p>0.05$) to the rodent data for BMD(L) (p-values for the Pearson X^2 goodness of fit test shown in Tables A-3 through A-5).

In Table A-3, the BMD(L) and 8-hr TWA estimates are based on the IT, PA, or short-term inhalation exposure studies of SWCNTs or MWCNTs with continuous response measures. Lung responses in rodents were evaluated at 32 to 60 days after first exposure. Rodent dose is the administered (IT or PA) or estimated deposited dose (inhalation). The BMR is the specified adverse lung response at 1.1 standard deviations above the estimated rodent control mean response (i.e., alveolar connective tissue thickness or amount of hydroxyproline) (as explained in Section A.2.3.2). Considerably higher 8-hr TWA concentrations are estimated based on the endpoint of lung hydroyxproline amount [Muller et al. 2005] compared to those based on the alveolar connective tissue thickness endpoint, which is a more sensitive (earlier) indicator of fibrosis [Mercer et al. 2008].

In Table A-4, the BMD(L) and 8-hr TWA estimates are based on the IT, PA, or short-term inhalation exposure studies of SWCNT or MWCNT with dichotomous response measures. Lung responses were evaluated 90 days after the first exposure. Rodent dose is the administered dose (IT or PA) or estimated deposited dose (inhalation). The BMR is the 10% excess risk of the specified adverse lung response. Although Lam et al. [2005] report dose-response data for three different preparations of SWCNT (containing either 2% Fe, 27% Fe, or 26% Ni), the BMD(L) and 8-hr TWA estimates are provided only for the SWCNT with 2% Fe, which was the only dataset of the three reported by Lam et al. [2005] that was adequately fit by the BMD model (Table A-4).

Table A-5 provides the BMD(L) estimates and 8-hr TWA estimates based on the two subchronic inhalation studies of MWCNTs, which report dichotomous response measures. Lung responses were evaluated at the end of the 13-week (91 d) exposure period. Rodent dose is either the total deposited lung dose or the retained lung dose at the end of exposure. The BMR is the 10% excess risk of the specified adverse lung response. As expected, the estimates based on deposited lung dose are lower than

those based on the retained lung dose because the assumption of no clearance in the deposited lung dose results in a lower estimated 8-hr TWA concentration to attain the BMD(L) lung burdens. The estimates for MWCNT (with 9.6% Al_2O_3) based on the rat granulomatous inflammation response are lower than those for MWCNT (Baytubes®) (with 0.53% Co) based on the rat focal septal thickening response.

Table A-6 provides estimates of the 8-hr TWA concentrations associated with 0.1, 1, and 10% excess risk, based on the subchronic inhalation studies and estimated deposited lung dose (i.e., assuming no clearance of CNT from the lungs). In Table A-7, the animal and human benchmark dose estimates and equivalent working lifetime 8-hr TWA concentrations associated with grade 2 (slight/mild) or higher lung responses in the subchronic inhalation studies, also based on estimated deposited lung dose. As expected, higher BMD(L)s and 8-hr TWA concentrations are estimated from the histopathology grade 2 or higher lung responses (Table A-6) compared to those estimated from histopathology grade 1 (minimal) or higher (Table A-5) because more animals developed the grade 1 or higher response at a given dose. That is, histopathology grade 1 or higher is a more sensitive response.

A.3.2 Comparison of short-term and subchronic dose-response data

Two studies of MWCNTs (Baytubes) provided an opportunity to examine the effect of dose-rate on the same lung response measured at the same time point. Wistar rats were exposed by inhalation for either one 6-hr day [Ellinger-Ziegelbauer and Pauluhn 2009] or 13 weeks (6 hr/d, 5 d/wk) [Pauluhn 2010a]. Lung responses were examined in both studies at 13 weeks after the first exposure. Histopathology severity scores for fibrosis (focal septal thickening) were available for each study. The number of male rats with focal septal thickening (of minimal or higher grade) and the respective exposure concentrations are as follows: Ellinger-Ziegelbauer and Pauluhn [2009]: 1, 0, and 6 rats (6 total per group) at 0, 11.0, and 241.3 mg/m^3; and Pauluhn [2010a, extended by personal communication]: 0, 0, 9, 10, 10 rats (10 total per group) at 0, 0.1, 0.45, 1.62, and 5.98 mg/m^3. The dose metric used for this comparison was the deposited lung

dose, estimated from MPPD 2.0 [CIIT and RIVM 2006] based on the particle size data (MMAD and GSD) and the exposure conditions reported in each study.

To evaluate whether these data [Pauluhn 2010a; Ellinger-Ziegelbauer and Pauluhn 2009] can be described by the same dose-response relationship, a multistage (polynomial degree 2) model [EPA 2010] was fit to the combined data (Figure A-4). This model provided adequate fit to the data (p=0.37), suggesting that these data can be described by the same dose-response model using the estimated total deposited lung dose, regardless of the dose rate (i.e., obtained in one day vs. over 90 days). This finding is consistent with the impaired clearance and biopersistence of the MWCNT in the rat lungs [Pauluhn 2010a].

A.4 Discussion

NIOSH conducted a quantitative risk assessment of CNTs by evaluating dose-response data of early-stage adverse lung effects in rats and mice exposed to several types of SWCNT or MWCNT (with different metal contaminants) by several routes of exposure (inhalation, PA or IT), and duration of exposure (single day or subchronic) and post-exposure (up to 4 months). Due to the different study designs and response endpoints used in the rodent studies, it is not possible to quantify to what extent the differences in the risk estimates are due to CNT material versus other study differences. Some evidence indicates CNT with certain metals (nickel, 26%) and with higher metal content (18% vs. 0.2%Fe) are more toxic and fibrogenic [Lam et al. 2004; Shvedova et al. 2005, 2008]. However, both unpurified and purified (low metal content) CNT were associated with early-onset and persistent pulmonary fibrosis and other adverse lung effects at relatively low mass doses. For example, the LOAELs for MWCNT (containing either 9.6% Al_2O_2 or 0.5%Co) were 0.1 and 0.4 mg/m^3 in 13-week inhalation studies in rats [Ma-Hock et al. 2009 and Pauluhn 2010a, respectively], which are more than an order of magnitude lower than the LOAEL of 7 mg/m^3 for ultrafine carbon black in the same species and study design [Elder et al. 2005]. No chronic animal studies or

epidemiological studies of workers producing or using CNT have been published in the literature.

The subchronic inhalation studies of MWCNT in rats [Ma-Hock et al. 2009; Pauluhn 2010a] provide the best data currently available to estimate the risk of occupational exposure to CNT. The IT, PA, and short-term inhalation exposure studies provide additional dose-response data for SWCNT [Lam et al. 2004; Shvedova et al. 2005, 2008] and for other MWCNT with different metal contaminants [Muller et al. 2005; Ellinger-Ziegelbauer and Pauluhn 2009; Porter et al. 2010] (Table A-1). Since there was no subchronic inhalation study of SWCNT available, the IT, PA, or short-term inhalation exposure studies provide the only dose-response data available for SWCNT. Although there is additional uncertainty in estimating long-term risk from these short-term studies, these studies provide supporting data to those of the subchronic studies.

A strength of the subchronic studies is that they are based on repeated exposures (13 weeks) by inhalation, the same route of exposure as encountered by workers exposed to airborne CNT. However, there is some uncertainty about the deposited and retained dose in the rat lungs. In the PA or IT studies, the administered lung dose is known, although the pattern of lung deposition (especially for IT) may differ from that of inhalation. The subchronic inhalation studies and some of the PA studies include multiple doses, which can provide better information about the shape of the dose-response relationship. However, in the subchronic studies, steep dose-response relationships were observed, reaching 100% of animals with early-stage adverse lung effects (Figure A-1). Dose groups with maximum (or near maximum) response provide little information for estimating a BMD.

All of these studies reported inflammatory and fibrotic lung effects of relevance to humans. These adverse lung effects were relatively early-stage; yet, these effects were not reversible after exposure ended. In the studies with multiple post-exposure follow-up times, the amount of pulmonary fibrosis persisted or progressed with longer follow-up [Shvedova et al. 2005; 2008; Mercer et al. 2008; Porter et al. 2010; Pauluhn 2010a].

One of the measures of pulmonary fibrosis, alveolar epithelial cell thickness (due to collagen deposition), was used in the development of the U.S. EPA ozone standard. This response endpoint was selected by EPA as the adverse lung response for cross-species dose-response extrapolation because it indicates "fundamental structural remodeling" [EPA 1996; Stockstill et al. 1995].

The excess risk estimates based on the subchronic and short-term studies of MWCNT and SWCNT suggest that workers are at >10% excess risk of developing early-stage adverse lung effects (pulmonary inflammation and fibrosis, minimal or higher grade) if exposed for a working lifetime at the current limit of quantitation (LOQ) of 7 µg/m^3 based on NIOSH Method 5040 for measuring the airborne concentration of CNT (see Section 5; Tables A-3 through A-7). Working lifetime airborne concentration (8-hr TWA) estimates of 0.51-4.2 (0.19-1.9) µg/m^3 (MLE and 95% LCL estimates, respectively) were associated with a 10% excess risk based on the subchronic inhalation studies (Table A-5). Lower exposure concentrations are estimated to be associated with lower risks of early-stage adverse lung effects (Table A-6).

Although there are uncertainties and limitations in these animal studies, the weight of the evidence supports the health-based need to reduce exposures below 7µg/m^3 and to develop more sensitive measurement methods. Based on the excess risk estimates of early-stage adverse lung effects in the animal studies and on the technical feasibility of measuring airborne CNT in the workplace (LOQ of 7 µg/m^3), NIOSH recommends reducing exposures to CNT below a recommended exposure limit (REL) of 7µg/m^3 (8-hr TWA). NIOSH prefers to recommend health-based RELs to protect workers that have no or very low residual risk, even if the risk is of early-stage adverse effects associated with the equivalent workplace exposure. However, the technical feasibility of measuring and controlling exposures can also influence the setting of the REL, as for CNT. These risk estimates indicate the need for research to develop more sensitive measurement methods for airborne CNT in the workplace, effective exposure control, and consideration of additional risk management measures such as the use of respirators

and other personal protective equipment and medical screening (Chapter 6; Appendix B).

A.4.1 The use of short-term data to predict longer-term response

Several factors suggest that in the absence of chronic data these short-term and subchronic animal data may be reasonable for obtaining initial estimates of the risk of human noncancer lung effects from exposure to CNT. First, some fraction of CNT that deposit in the lungs is likely to be biopersistent based on studies in animals [Muller et al. 2005; Deng et al. 2007; Elgrabli et al. 2008b; Mercer et al. 2009; Pauluhn 2010a,b] and studies of other poorly-soluble particles in human lungs [ICRP 1994; Kuempel et al. 2001]. Second, the pulmonary fibrosis developed earlier and was of equal or greater severity than that observed from exposure to the same mass dose of other inhaled particles or fibers (silica, carbon black, asbestos) in the same study [Shvedova et al. 2005; Muller et al. 2005]. Third, the adverse lung responses persisted or progressed up to 90 days post-exposure after a single- or few-day exposure to SWCNT or MWCNT [Lam et al. 2004; Muller et al. 2005; Shvedova et al. 2005, 2008; Ellinger-Ziegelbauer and Pauluhn 2009; Porter et al. 2010]. The alveolar interstitial (septal) thickening observed following a 13-week inhalation exposure to MWCNTs (Baytubes®) also persisted or progressed in rats examined up to 6 months after the end of exposure [Pauluhn 2010a].

A comparison of data from 1-day and 13-week inhalation exposures in rats [Ellinger-Ziegelbauer and Pauluhn 2009; Pauluhn 2010a], indicate that the dose-response relationship was the same despite the differences in dose-rate (Figure A-4). This finding indicates that it may be reasonable to assume that the dose-response relationships for the IT, PA, and short-term inhalation exposure studies would be consistent with the subchronic study results if the same response is examined at the same time point, although additional study is needed to confirm this finding.

A.4.2 Metal content

There are limited data to evaluate the role of CNT type and metal content on the lung responses in rats and mice, and comparisons between studies are difficult due to the differences in study design. In one of the few studies to investigate CNT with different metal content, Lam et al. [2004] reported lung granuloma and inflammation responses in mice administered IT doses of SWCNT containing either 2% Fe, 27% Fe, or 26% Ni. The number of mice developing granulomas in the 0.1 and 0.5 mg dose groups (5 mice per group) were, respectively: 2 and 5 (2% Fe); 5 and 5 (27% Fe); and 0 and 5 (including 3 mice that died in the first week) (26% Ni). However, due to the sparse data and the steep dose-response relationship, only the SWCNT containing 2% Fe was adequately fit by the BMDS model. The high mortality rate in mice exposed to the Ni-containing SWCNTs suggests this material is highly toxic. The greater response proportion in the mice exposed to 0.1 mg SWCNT with 27% Fe compared to rats exposed to the same dose of SWCNT with 2% Fe suggests that the CNT with the higher Fe content is more toxic than CNT with lower Fe content. In Shvedova et al. [2005; 2008], higher iron content was also associated with greater lung response and thus lower BMD(L). The BMD(L) estimates for SWCNT with 18% Fe were lower than those for SWCNT with 0.2% Fe (Table A-3), even though the post-exposure time was longer (60 vs.28 days) for the 0.2% Fe SWCNT [Shvedova et al. 2005, 2008]. However, all types of CNT (including SWCNT and MWCNT, whether purified or unpurified, and with various types and percentages of metals) were of similar of greater potency (i.e., similar or greater lung response at the same mass dose) in these animal studies than other types of particles or fibers tested (asbestos, silica, ultrafine carbon black) [Lam et al. 2004; Muller et al. 2005; Shvedova et al. 2005, 2008; Elder et al. 2005].

A.4.3 Estimated lung dose

The BMD(L) estimates based on the estimated retained lung dose in rats are lower than those based on the estimated deposited lung dose (Table A-5). This is because the retained dose estimates allows for some lung clearance to occur over the 13-week

exposure in rats, and the lower dose estimate is therefore associated with a given response proportion. The human-equivalent BMD(L)s estimates based on retained dose are also lower because they are proportional to the rat BMD(L)s (i.e., calculated based on the ratio of the human to rat lung surface area). However, the working lifetime 8-hr TWA concentrations based on the estimated retained lung doses are higher than those based on the estimated deposited lung dose. This is because the retained dose estimates (which assume some particle clearance from workers' lungs during the 45-years of exposure), require a higher inhaled airborne concentration to reach the human-equivalent BMD(L) lung dose.

The estimated deposited lung dose of CNT (assuming no clearance) may overestimate the actual lung dose, based on analogy to lung clearance of spherical particles [ICRP 1994] and fibers [Sturm and Hofmann 2009], and given the short-term lung kinetic data of CNT in animals [Muller et al. 2005; Deng et al. 2007; Elgrabli et al. 2008b; Mercer et al. 2009; Pauluhn 2010a,b]. On the other hand, the estimated retained lung dose of CNT, based on models of poorly-soluble spherical particles, may underestimate the actual lung burden given the reduced lung clearance observed in rats inhaling MWCNT (Baytubes®) compared to the same mass doses of other poorly soluble particles [Pauluhn 2010b]. Thus, while there is uncertainty in the deposition and retention of CNT in the animal and human lungs, the deposited and retained lung dose estimates reported in this risk assessment may represent reasonable upper and lower bounds of the actual lung doses.

A.4.4 Strengths and Limitations

As in any risk assessment, the limitations in CNT risk assessment are the areas of uncertainty. Some of these uncertainties are consistent with those in other chemical and particle or fiber risk assessments based on animal data. These include uncertainty about whether the human and animal lung responses would be equal at a given equivalent dose; and whether there are differences in the inter-individual responses (e.g., the variability in response in human populations is often greater than that

estimated from controlled experimental animal data). In any CNT risk assessment, there is greater uncertainty in the estimated lung dose of respirable CNT than there is for spherical airborne particles, for which lung dosimetry models have been developed and validated. The influence of particle characteristics (e.g., shape and density) on the inhalability and deposition of CNT in human respiratory tract, and on the clearance or biopersistence of CNT have not been evaluated. However, the available data on the aerodynamic size of CNT provides an initial estimate (based on validated models for spherical particles) of the deposited mass fraction of airborne CNT in the human respiratory tract including the alveolar (gas exchange) region. The clearance rate of CNT from the lungs may be more uncertain, as animals studies indicate that CNT clearance becomes impaired in rat lungs at lower mass doses than for larger particles of greater density [Pauluhn 2010a,b]. The NIOSH risk assessment helps to address this uncertainty by providing risk estimates based on the range of possible lung dose estimates from assuming normal clearance to assuming no clearance of the deposited CNT. This approach also provides a framework for introducing improved dose estimates when validated lung dosimetry models for CNT become available.

The NIOSH risk assessment using BMD modeling methods provides a standardized method for risk estimation. In contrast, the NOAEL-based approaches do not estimate risk but assume safe exposure or zero risk below the derived OEL. BMD modeling also takes appropriate statistical account of sample size and uses all of the dose-response data rather than only a single dose. The BMD modeling options in these CNT data were limited due to sparse data, and dose groups with 100% response (observed in the subchronic inhalation studies) contribute little information to the BMD estimation. A common challenge in risk assessment is defining a biologically-relevant response for continuous endpoints, which was also encountered in this risk assessment. A standard practice of using a statistical definition of the benchmark response was used here for the continuous BMD estimation in the absence of data on the functional significance of the early-stage pulmonary inflammation and fibrotic responses.

For CNT, as with other chemicals, there is uncertainty in whether a NOAEL or a BMD(L) from a short-term or subchronic study in animals would also be observed in a chronic study. For example, in the Pauluhn [2010a] study, 0.1 mg/m^3 was the NOAEL based on subchronic inhalation exposure in rats, but there were indications that lung clearance overloading was already occurring (i.e., retention half-time about two-fold higher than normal) [Pauluhn 2010a,b]. Thus, it is not known whether chronic exposures to 0.1 mg/m^3 might result in adverse effects that were not observed during subchronic exposure. The same uncertainty applies to the other studies used in this risk assessment, especially the studies with shorter-term exposure or follow-up compared to the subchronic studies.

In the absence of epidemiological data for CNT, the two subchronic inhalation studies of two types of MWCNT, in addition to the short-term studies of SWCNT and MWCNT, provide the best available data to develop initial estimates of the risk of early-stage adverse lung responses associated with exposure to CNT. Some of these studies provide data comparing the potency of CNT with that of other particles or fibers for which animal and human data are available on the long-term adverse health effects. These studies show that on a mass basis, CNT had equal or greater potency (pulmonary inflammation or fibrosis response at a given mass dose) than did ultrafine carbon black, crystalline silica, or chrysotile asbestos [Lam et al. 2004; Muller et al. 2005; Shvedova et al. 2005]. These comparative potency findings reduce the uncertainty about whether CNT may be a respiratory hazard and support the need to reduce CNT exposures to low airborne respirable mass concentrations to reduce the risk of chronic lung diseases in workers. In addition, strength of the animal evidence is that dose-response data are available for different types of CNT, including SWCNT or MWCNT, either purified or unpurified (containing different types and amounts of metal). Although a formal comparison of the potency of the different CNT is not feasible due to differences in study design, these studies consistently show that relatively low mass doses of CNT are associated with early-stage adverse lung effects in rats and mice. Consequently, the human-equivalent benchmark dose and working lifetime exposure estimates derived from these studies are also relatively low on the mass basis. That is,

the excess risk estimates of early-stage adverse lung responses to CNT generally indicate >10% excess risk (lower 95% confidence limit estimates) at the upper LOQ of the current measurement method (NIOSH Method 5040) regardless of the CNT type or purification.

The response endpoints in the animal studies of CNT are all relatively early-stage, and while the responses were persistent or progressive after the end of exposure in some studies, there was no information on whether these responses were associated with adverse functional effects. It is expected that exposure limits derived from early response data would be more protective than those based on frank adverse effects. On the other hand, there is considerable uncertainty about the chronic adverse health endpoints including cancer due to the lack of chronic studies.

A.5 Conclusions

These risk estimates were developed using benchmark dose methods applied to rodent dose-response data of adverse lung effects following subchronic or short-term exposure to various SWCNT and MWCNT. The subchronic inhalation studies of MWCNT provide the best data currently available to estimate the risk of occupational exposure to CNT. The IT, PA, and short-term inhalation exposure studies of SWCNT and MWCNT support the findings from the subchronic studies. In the absence of validated lung dosimetry models for CNT, lung doses were estimated assuming either deposited or retained lung dose in animals or humans. These findings indicate that workers are at risk of developing adverse lung effects including pulmonary inflammation and fibrosis if exposed to CNT over a working lifetime. Based on the two subchronic inhalation studies for two types of MWCNT (with different metal content), working lifetime exposures of 0.2 - 2 $\mu g/m^3$ (8-hr TWA concentration) are associated with a 10% excess risk of early-stage adverse lung effects (95% LCL estimates). These values are below the upper LOQ (7 $\mu g/m^3$) of NIOSH Method 5040 for measuring the respirable mass concentration of CNT in air as an 8-hr TWA. Risk estimates based on the short-term studies for SWCNT and MWCNT are also generally below the LOQ of the analytical

method, regardless of whether the CNT was purified or unpurified (with different types and amounts of metal). This risk assessment was used in developing a NIOSH recommended exposure limit (REL) for CNT (Section 5).

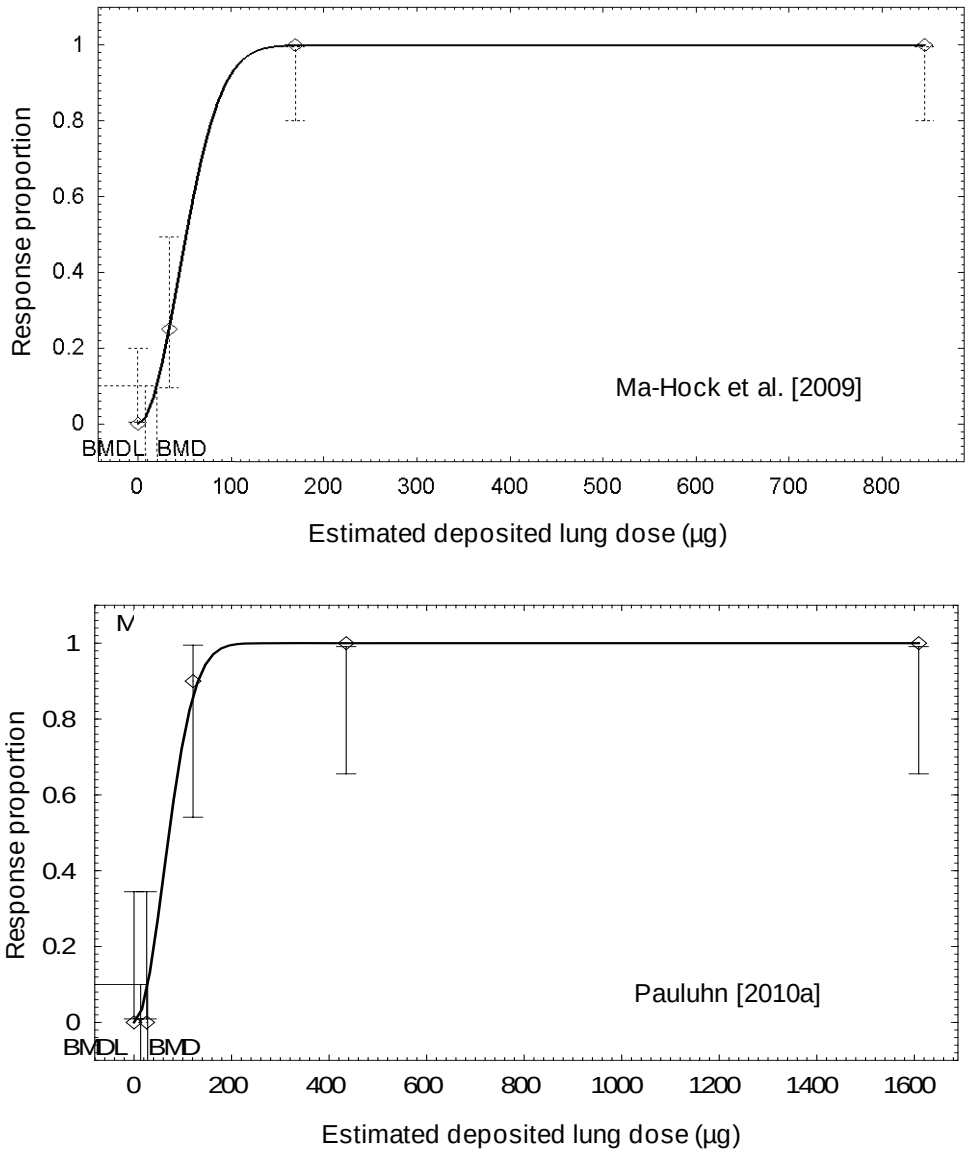

Figure A-1. Benchmark dose model (multistage, polynomial degree 2) fit to rodent dose-response data from the two subchronic inhalation studies of MWCNT in rats: Ma-Hock et al. [2009], response: granulomatous inflammation; Pauluhn [2010a], response: alveolar septal thickening, minimal or greater. P-values are 0.13 and 0.65, respectively, for Ma-Hock et al. [2009] and Pauluhn [2010a].

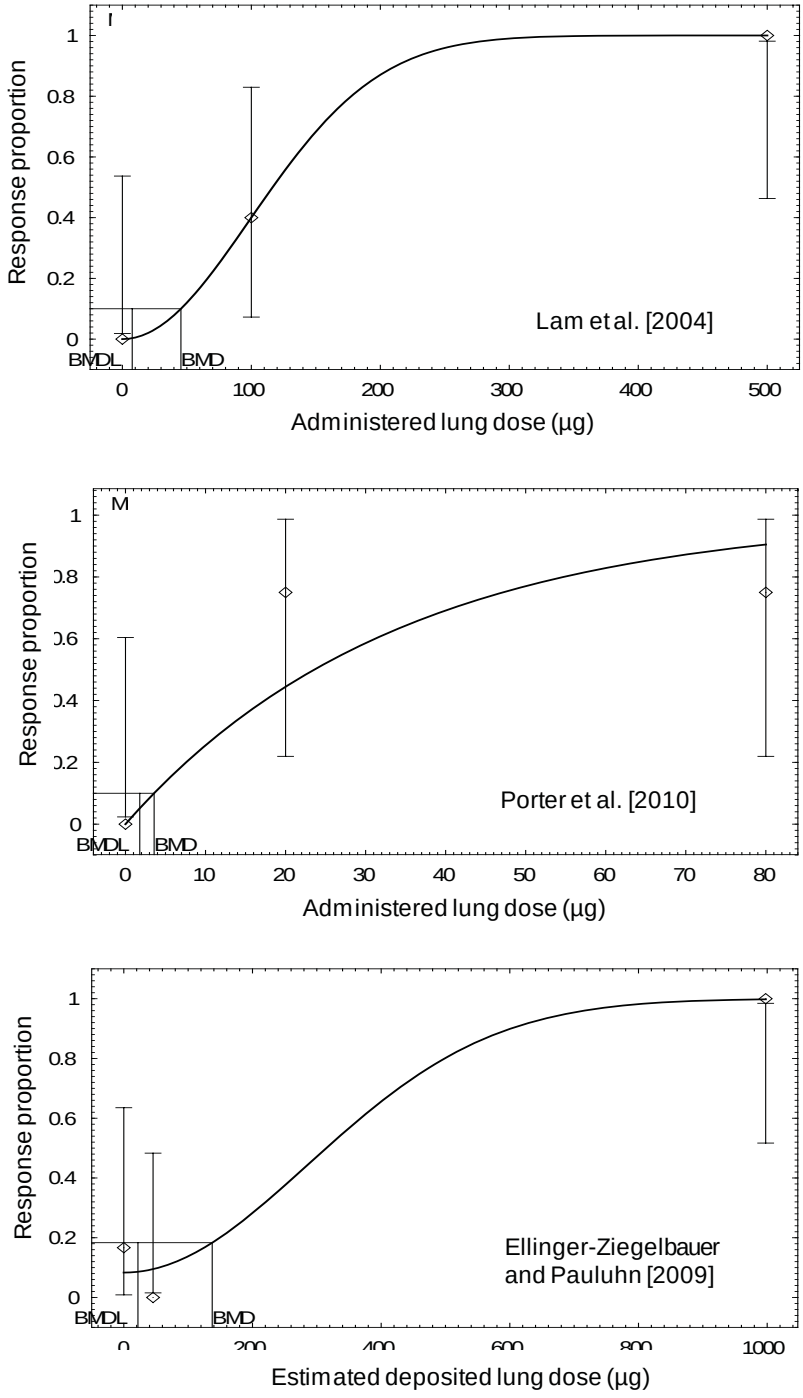

Figure A-2. Benchmark dose model (multistage, polynomial degree 2) fit to rodent dose-response data from short-term studies with dichotomous response: Ellinger-Ziegelbauer and Pauluhn [2009] (MWCT, rat, short-term inhalation; response: alveolar interstitial thickening, minimal or higher); Porter et al. [2010] (MWCNT, mouse, pharyngeal instillation; response: fibrosis severity score, grade 6 or higher); Lam et al. [2004] (SWCNT, mouse, intratracheal instillation; response: lung granulomas). P-values: 0.35, 0.31, and 0.052 for Lam et al. [2004], Porter et al. [2010], and Ellinger-Ziegelbauer and Pauluhn [2009], respectively.

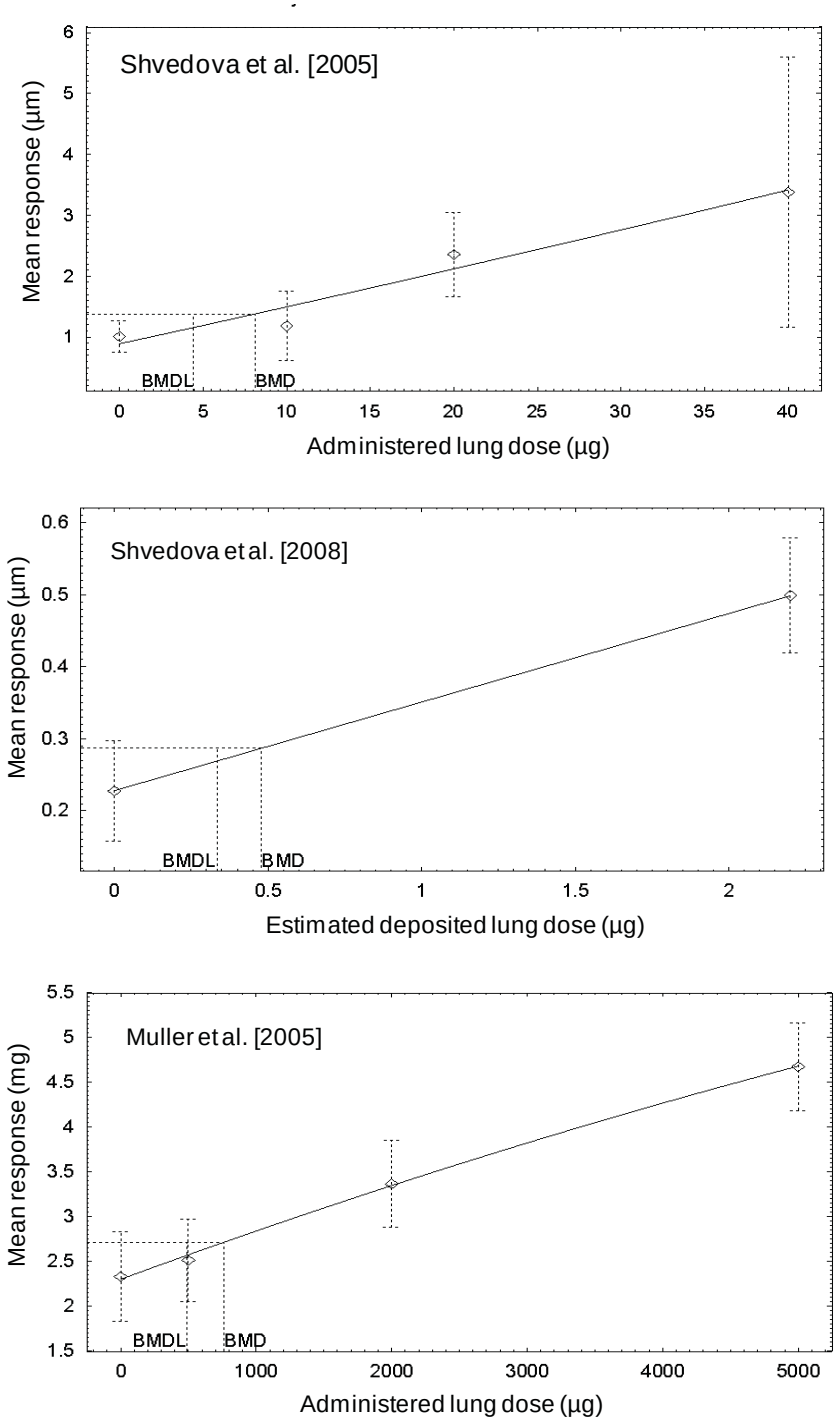

Figure A-3. Benchmark dose model fit to rodent dose-response data from short-term studies with continuous response: Shvedova et al. [2005] (SWCNT, mouse, pharyngeal aspiration; response: alveolar connective tissue thickening); Shvedova et al. [2008] (SWCNT, mouse, inhalation; response: alveolar connective tissue thickening); Muller et al. [2005] (MWCNT, intratracheal instillation, rat; response: hydroxyproline amount). Benchmark response level: 1.1 standard deviations about the control mean response. P-values: 0.089, not applicable (linear), and 0.67 for Shvedova et al. [2005], Shvedova et al. [2008], and Muller et al. [2005], respectively.

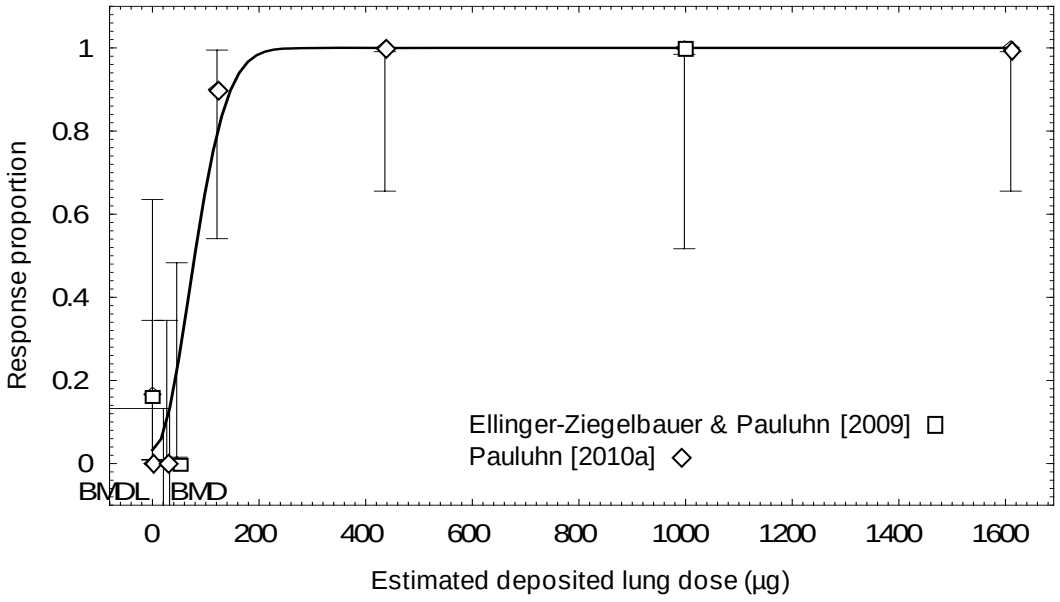

Figure A-4. Dose-response for estimated deposited lung dose of MWCNT (Baytubes) and early-stage pulmonary fibrosis (proportion of rats with minimal or greater focal interstitial thickening) examined at 13 weeks, following either a 1-day [Ellinger-Ziegelbauer and Pauluhn 2009] or 13-week inhalation exposure [Pauluhn 2010a]. Deposited lung dose estimated using aerodynamic size data in MPPD2 [CIIT and RIVM 2007]. Dose groups include n=10 [Pauluhn 2010a] or n=6 [Ellinger-Ziegelbauer and Pauluhn 2009]. Data were fit with a multistage (polynomial degree 2) model in BMDS 2.2 [EPA 2010]. Error bars are the 95% confidence limits.

Table A-1. Rodent study information

Rodent study	CNT type and main metal component	Species, strain, gender	Route of exposure [a]	Number of animals per dose group	Dose (& exposure duration, if inhalation)	Post-exposure days	Lung response
Lam et al. [2004]	SWCNT Fe 2.0%	Mouse, B6C3F1, male	IT	5	0, 0.1, or 0.5 mg	90	Granuloma[b]
Shvedova et al. [2005]	SWCNT Fe 0.2%	Mouse, C57BL/6, female	PA	6 (28 d) 3 (60 d)	0, 10, 20, 40 µg	1, 3, 7, 28, 60	Alveolar connective tissue thickness[c]
Muller et al. [2005]	MWCNT Al 2%, Co 0.5%, Fe 0.5%	Rat, Sprague-Dawley, female	IT	5	0, 0.5, 2, 5 mg	28 & 60	Hydroxyproline amount[c]
Shvedova et al. [2008]	SWCNT Fe 18%	Mouse, C57BL/6, female	Inhal	5	5 mg/m^3 (5 hr/d, 4 d)	1, 7, 28 (after 4d exposure)	Alveolar connective tissue thickness[c]
Ma-Hock et al. [2009]	MWCNT Al_2O_3 9.6%	Rat, Wistar (Crl:WI), male & female	Inhal	20 (10 each gender)	0, 0.1, 0.5, 2.5 mg/m^3 (6 hr/d, 5 d/wk, 13 wk)	1	Granulomatous inflammation (minimal or greater) [b]

Table A-1 (Continued). Rodent study information

Rodent study	CNT type and main metal component	Species, strain, gender	Route of exposure [a]	Number of animals per dose group	Dose (and exposure duration, if inhalation)	Post-exposure days	Lung response
Porter et al. [2010]	MWCNT Fe 0.3%	Mouse, C57BL/6J, male	PA	4	0, 10, 20, 40, 80 µg	7, 28, 56	Fibrosis severity (score 6 or greater) [b]
Ellinger-Ziegelbauer & Pauluhn [2009]	MWCNT Co 0.5%	Rat, Wistar (HsdCpb: WU), male	Inhal	6	0, 11, & 241 mg/m^3 (6r/d, 1 d)	90	Focal septal thickening (minimal or greater) [b]
Pauluhn [2010]	MWCNT Co 0.5%	Rat, Wistar (HsdCpb: WU), male	Inhal	10	0, 0.10, 0.45, 1.62, 5.98 mg/m^3 (6 hr/d, 5 d/wk, 13 wk)	1, 28, 91, 182	Focal septal thickening (minimal or greater) [b]

[a] Intratracheal instillation (IT); pharyngeal aspiration (PA); inhalation (inhal).
[b] Dichotomous response.
[c] Continuous response.

Table A-2. CNT particle size and alveolar deposition fraction in rodent and human

Study	Particle Size Information	Human DF_{alv}[a] and MMAD (GSD) used	Rodent DF_{alv} (same MMAD and GSD)
Lam et al. [2004]	Not reported; same SWCNT source as Shvedova et al. [2005]. [assume same MMAD (GSD) as Shvedova et al. 2008]	0.076 3.5 (2.14)	ad [e]
Shvedova et al. [2005]	1-4 nm width (primary particles) [assume same MMAD (GSD) as Shvedova et al. 2008]	0.076 3.5 (2.14)	ad
Muller et al. [2005]	9.7 nm width; 5.9 µm length (primary particles) [assume same MMAD (GSD) as Ma-Hock et al. 2009]	0.099 1.2 (2.7)	ad
Shvedova et al. [2008]	0.8-1.2 nm width; 100-1000 nm length (primary particles). 4.2 µm mass mode diameter. 240 nm count mode diameter 3.5 µm MMAD (2.14 GSD) [d]	0.076 3.5 (2.14)	0.01 [c]
Ma-Hock et al. [2009]	1.5 (3.6); 1.2 (2.7); 0.8 (2.8) µm MMAD (GSD) at 0.1, 0.5, and 2.5 mg/m^3, respectively (median of 3 values at each concentration). Primary particles: 5-15 nm width; 0.1-10 µm length	0.099 1.2 (2.7)	0.072 [b]
Porter et al. [2010]	1.5 µm MMAD [GSD not reported; assume 2]; count mean width (49 nm; 13.4 SD); median length 3.86 µm (1.94 GSD)	0.10 1.5 (2)	ad
Ellinger-Ziegelbauer & Pauluhn [2009]	2.9 (1.8) & 2.2 (2.6) µm MMAD (GSD) at 11 & 241 mg/m^3, respectively. [Same MWCNT as Pauluhn [2010a]; assumed same size & deposition fraction].	0.086 2.74 (2.11)	0.046 [b]
Pauluhn [2010a]	3.05 (1.98); 2.74 (2.11); 3.42 (2.14) µm MMAD (GSD) at 0.4, 1.5, and 6 mg/m^3, respectively. Primary particles: ~10 nm width; 200-1,000 nm length	0.086 2.74 (2.11)	0.046 [b]

[a] MPPD 2.0 human; Yeh and Schum deposition model; 9.6 m^3/8 hr d (20 L/min, or 1143 ml tidal volume at 17.5 breaths/min); inhalability adjustment; assumed unit density.

[b] MPPD 2.0 rat; 0.21 L/min or 2.45 ml tidal volume (assuming 300g male and female rats) [Ma-Hock et al. 2009]; and 0.25 L/min or 2.45 ml tidal volume (369 g male rats) [Pauluhn 2010] [EPA 1995; 2006]; inhalability adjustment; assumed unit density.

[c] Raabe et al. [1988]: mouse DF_{alv} interpolated from values in Table 2 of Raabe et al. [1988].

[d] MMAD and GSD in Shvedova et al. [2008] were estimated from data reported in Baron et al. [2008] [personal communication from B. Chen to E. Kuempel, August 4, 2009].

[e] ad – administered dose by intratracheal instillation or pharyngeal aspiration.

Table A-3. Benchmark dose estimates[a] and associated human working lifetime airborne concentrations – Continuous response data in rats or mice exposed to SWCNT or MWCNT by IT, PA,[e] or short-term inhalation (dose metric: administered or estimated deposited lung dose)

Rodent study, CNT type, and response	Rodent		Human		Human working lifetime airborne concentration [b] ($\mu g/m^3$)	
	BMD [c, d] (µg/lung)	BMDL (µg/lung)	BMD (mg/lung)	BMDL (mg/lung)	BMC	BMCL
IT or PA						
Muller et al. [2005] - MWCNT Hydroxyproline amount (at 60 d) in rats	760	486	194	124	18	12
Shvedova et al. [2005] – SWCNT (0.2% Fe) Alveolar connective tissue thickness (at 60 d) in mice	8.1	4.4	15	8.2	1.8	1.0
Inhalation (5 hr/d, 4 d)						
Shvedova et al. [2008] – SWCNT (18% Fe) Alveolar connective tissue thickness (at 32 d) in mice	0.48	0.33	0.89	0.62	0.11	0.075

[a] Benchmark response level: 1.1 standard deviations above estimated control mean response [Crump 1995; EPA 2010]; associated with a 10% increase in abnormal response (assumed to be greater than the 99th percentile of the distribution of control responses).

[b] 8-hr time weighted average (TWA) concentration associated with the human-equivalent BMD(L)s; BMC: maximum likelihood estimate of the benchmark concentration; BMCL: 95% lower confidence limit of the BMC.

[c] BMD: estimated benchmark dose (maximum likelihood estimate): BMDL: estimated 95% lower confidence limit of the BMD; polynomial (degree 2) model [EPA 2010].

[d] P-values for the rodent dose response models: 0.67 for Muller et al. [2005], 0.089 for Shvedova et al. [2005], and not applicable (linear) for Shvedova et al. [2008], respectively.

[e] IT: intratracheal instillation [Muller et al. 2005]; PA: pharyngeal aspiration [Shvedova et al. 2005].

Table A-4. Benchmark dose estimates[a] and associated human working lifetime airborne concentrations – Dichotomous response data in rats or mice exposed to SWCNT or MWCNT by IT, PA,[e] or short-term inhalation(dose metric: administered or estimated deposited lung dose)

Rodent study, CNT type, and response	Rodent		Human		Human working lifetime airborne concentration ($\mu g/m^3$) [b]	
	BMD [c, d] (µg/lung)	BMDL (µg/lung)	BMD (mg/lung)	BMDL (mg/lung)	BMC	BMCL
IT or PA						
Lam et al. [2004] – SWCNT (2% Fe) Granuloma (at 90 d) in mice	45	7.6	84	14	10	1.7
Porter et al. [2010] – MWCNT Fibrosis – grade 4 or greater (at 56 d) in mice	3.6	1.8	6.6	3.3	0.61	0.31
Inhalation (6 hr/d, 1 d)						
Ellinger-Ziegelbauer & Pauluhn [2009] – MWCNT (0.5% Co) in rats Focal septal thickening – minimal or greater (at 91 d)	137	22	35	5.6	3.8	0.60

[a] Benchmark response level: 10% excess (added) risk in exposed animal [EPA 2010].
[b] 8-hr time weighted average (TWA) concentration associated with the human-equivalent BMD(L)s; BMC: maximum likelihood estimate of the benchmark concentration; BMCL: 95% lower confidence limit of BMC.
[c] BMD: estimated benchmark dose (maximum likelihood estimate): BMDL: estimated 95% lower confidence limit of the BMD; multistage (polynomial degree 2) model [EPA 2010].
[d] P-values for the rodent dose-response models: 1.0 for Lam et al. [2004]; 0.15 for Porter et al. [2010]; 0.28 for Ellinger-Ziegelbauer & Pauluhn [2009].
[e] IT: intratracheal instillation [Lam et al. 2004]; PA: pharyngeal aspiration [Porter et al. 2010]; 1d, 6-hr inhalation exposure in Ellinger-Ziegelbauer and Pauluhn [2009].

Table A-5. Benchmark dose estimates [a] and associated human working lifetime airborne concentrations – Subchronic inhalation of MWCNT in rats (dose metric: estimated deposited or retained dose)

Rodent study and response [b]	Rodent		Human		Human working lifetime airborne concentration [c] ($\mu g/m^3$)	
	BMD [d, e] (µg/lung)	BMDL (µg/lung)	BMD (mg/lung)	BMDL (mg/lung)	BMC	BMCL
Deposited lung dose						
Ma-Hock et al. [2009] Granulomatous inflammation	21	8.1	5.4	2.1	0.51	0.19
Pauluhn [2010a] Focal septal thickening	28	14	7.2	3.5	0.77	0.38
Retained lung dose [f]						
Ma-Hock et al. [2009] Granulomatous inflammation	11	3.8	2.7	0.97	2.7	1.0
Pauluhn [2010a] Focal septal thickening	14	6.5	3.6	1.7	4.2	1.9

[a] Benchmark response level: 10% excess (added) risk in exposed animal [EPA 2010].
[b] Responses are histopathology severity grade 1 (minimal) or higher.
[c] 8-hr time weighted average (TWA) concentration associated with the human-equivalent BMD(L)s; BMC: maximum likelihood estimate of the benchmark concentration; 95% LCL: 95% lower confidence limit of the BMC.
[d] BMD: estimated benchmark dose (maximum likelihood estimate): BMDL: estimated 95% lower confidence limit of the BMD; multistage (polynomial degree 2) [EPA 2010].
[e] P-values for the rodent dose-response models: 0.99 for Ma-Hock et al. [2009] and 0.88 for Pauluhn et al. [2010a] (deposited dose); 1.0 for Ma-Hock et al. [2009] and 0.93 for Pauluhn et al. [2010a] (retained dose), respectively.
[f] Retained lung doses in rats and humans estimated using MPPD 2.0 model [CIIT and RIVM 2006] and aerodynamic particle sizes (MMAD, GSD) shown in Table A-2.

Table A-6. Human working lifetime airborne concentration (µg/m^3) associated with various excess risk levels – estimated from extrapolation of rodent dose-response model and estimated deposited lung dose [a]

Rodent study and response [b]	Excess Risk 10%		Excess Risk 1%		Excess Risk 0.1%	
	BMC	BMCL	BMC	BMCL	BMC	BMCL
Ma-Hock et al. [2009] Granulomatous inflammation	0.51	0.19	0.16	0.019	0.050	0.00197
Pauluhn [2010a] Focal septal thickening	0.77	0.38	0.24	0.042	0.075	0.0042

[a] BMDS model: multistage polynomial degree 2 [EPA 2010].
[b] Responses are histopathology severity grade 1 (minimal) or higher.

Table A-7. Benchmark dose estimates [a] and associated human working lifetime airborne concentrations – Grade 2 or higher severity of lung responses in subchronic inhalation of MWCNT in rats and estimated deposited lung dose

Rodent study and response	Rodent		Human		Human working lifetime airborne concentration [b] ($\mu g/m^3$)	
	BMD [c, d] (µg/lung)	BMDL (µg/lung)	BMD (mg/lung)	BMDL (mg/lung)	BMC	BMCL
Ma-Hock et al. [2009] Granulomatous inflammation – grade 2[e] or higher	44	29	11	7.4	1.0	0.69
Pauluhn [2010a] Focal septal thickening – grade 2[e] or higher	235	120	60	31	6.4	3.3

[a] Benchmark response level: 10% excess (added) risk in exposed animal [EPA 2010].
[b] 8-hr time weighted average (TWA) concentration associated with the human-equivalent BMD(L)s; BMC: maximum likelihood estimate of the benchmark concentration; 95% LCL: 95% lower confidence limit of the BMC.
[c] BMD: estimated benchmark dose (maximum likelihood estimate): BMDL: estimated 95% lower confidence limit of the BMD; multistage (polynomial degree 2) [EPA 2010].
[d] P-values for the rodent dose-response models: 0.67 for Ma-Hock et al. [2009] and 0.98 for Pauluhn et al. [2010a].
[e] Grade 2 is slight [Ma-Hock et al. 2009] or slight/mild [Pauluhn 2010a] severity based on histopathology (proportion of animals with that response).

APPENDIX B

Occupational Health Surveillance: Informing Decisions Concerning Medical Surveillance in Workplaces with Potential Exposure to CNT and CNF

Key Terms Related to Medical Surveillance

Occupational health surveillance involves the ongoing systematic collection, analysis, and dissemination of exposure and health data on groups of workers for the purpose of preventing illness and injury. Occupational health surveillance, which includes hazard and medical surveillance, is an essential component of an effective occupational safety and health program, [Harber et al. 2003; Baker and Matte 2005; NIOSH 2006; Wagner and Fine 2008; Trout and Schulte 2009] and NIOSH continues to recommend occupational health surveillance as an important part of an effective risk management program.

Hazard surveillance includes elements of hazard and exposure assessment.

- The hazard assessment involves reviewing the best available information concerning toxicity of materials; such an assessment may come from databases, texts, and published literature or available regulations or guidelines (e.g., from NIOSH or the Occupational Safety and Health Administration [OSHA]). Human studies, such as epidemiologic investigations and case series or reports, and animal studies may also provide valuable information. In most instances involving CNT there are limited toxicological data and a lack of epidemiologic data with which to make a complete hazard assessment.

- The exposure assessment involves evaluating relevant exposure route(s) (inhalation, ingestion, dermal, and/or injection), amount, duration, and frequency (i.e., dose), as well as whether exposure controls are in place and how protective they are. When data are not available, this will be a qualitative process.

Medical surveillance

Medical surveillance targets actual health events or a change in a biologic function of an exposed person or persons. Medical surveillance involves the ongoing evaluation of the health status of a group of workers through the collection and aggregate analysis of health data for the purpose of preventing disease and evaluating the effectiveness of intervention programs (primary prevention). NIOSH recommends the medical surveillance of workers when they are exposed to hazardous materials, and therefore are at risk of adverse health effects from such exposures. Medical screening is one form of medical surveillance that is designed to detect early signs of work-related illness in individual workers by administering tests to apparently healthy persons to detect those with early stages of disease or risk of disease; medical screening generally represents secondary prevention.

Medical surveillance is a second line of defense behind the implementation of engineering, administrative, and work practice controls (including personal protective equipment). Integration of hazard and medical surveillance is key to an effective occupational health surveillance program, and surveillance of disease or illness should not proceed without having a hazard surveillance program in place.

Planning and Conduct of Medical Surveillance

Important factors when considering medical surveillance include:

1. a clearly defined purpose or objective,
2. a target population which is clearly defined, and
3. the availability of testing modalities to accomplish the defined objective. Testing modalities may include such tools as questionnaires, physical examinations, and medical testing.

A clear plan should be established before a medical surveillance program is initiated. The plan should include:

1. a rationale for the type of medical surveillance,
2. provisions for interpreting the results,
3. presentation of the findings to workers and management of the affected workplace, and
4. implementation of all the other steps of a complete medical surveillance program [Harber et al. 2003].

The elements for conducting a medical surveillance program generally include the following:

1. An initial medical examination and collection of medical and occupational histories.

2. Periodic medical examinations at regularly scheduled intervals, including specific medical screening tests when warranted.

3. More frequent and detailed medical examinations as indicated on the basis of findings from these examinations.

4. Post-incident examinations and medical screening following uncontrolled or non-routine increases in exposures such as spills.

5. Worker training to recognize symptoms of exposure to a given hazard.

6. A written report of medical findings.

7. Employer actions in response to identification of potential hazards.

APPENDIX C

NIOSH Method 5040

Background

NIOSH Method 5040 is based on a thermal-optical analysis technique [Birch and Cary 1996] for organic and elemental carbon (OC and EC). The analysis quantifies total carbon (TC) in a sample as the sum of OC and EC. The Method was developed for measurement of diesel particulate matter (DPM) in occupational settings, but it is applicable to other types of carbonaceous aerosols. It is widely used for environmental and occupational monitoring.

For the thermal-optical analysis, a portion (typically a 1.5-cm^2 rectangular punch) of a quartz-fiber filter sample is removed and placed on a small quartz spatula. The spatula is inserted in the instrument's sample oven and the oven is tightly sealed. Quartz-fiber filters are required for sample collection because temperatures in excess of 850 °C are employed during the analysis. The thermal-optical analyzer is equipped with a pulsed diode laser and photo-detector that permit continuous monitoring of the filter transmittance. This optical feature corrects for the 'char' that forms during the analysis due to carbonization of some materials.

Thermal-optical analysis proceeds in inert and oxidizing atmospheres. In both, the evolved carbon is catalytically oxidized to carbon dioxide (CO_2). The CO_2 is then reduced to methane (CH_4), and CH_4 is quantified with a flame ionization detector (FID). The OC (and carbonate, if present) is first removed in helium, as the temperature is increased to a preset maximum. If sample charring occurs, the filter transmittance decreases as the temperature is stepped to the maximum. After the OC is removed in helium, an oxygen-helium mix is introduced to effect combustion of the remaining material. As the light-absorbing carbon (mainly EC and char) is oxidized from the filter the filter transmittance increases. The split between the OC and EC is assigned when the initial (baseline) value of the filter transmittance is reached. All carbon removed before the OC-EC split is considered organic, and that removed after the split is

considered elemental. If no charring occurs, the split is assigned prior to removal of EC. If the sample chars, the split is not assigned until enough light-absorbing carbon is removed to increase the transmittance to its initial value.

OC and EC results are reported as micrograms per square centimeter (µg/cm^2) of sample deposit. The total OC and EC on the filter are calculated by multiplying the reported values by the deposit area. Because only a portion of the sample is analyzed, it must be representative of the entire deposit. Thus, a homogeneous deposit is assumed. The entire filter must be analyzed (in portions if a 37 mm filter is used) if the filter deposit is uneven.

Method Evaluation

The reported accuracy of NIOSH 5040 is based on analysis of TC in different sample types. Accuracy was based on TC because there is no analytical standard for determining the OC-EC content of a complex carbonaceous aerosol. In the method evaluation, five different organic compounds were analyzed to examine whether the instrument response is compound dependent. Linear regression of the data (43 analyses total) for all five compounds gave a slope and correlation coefficient (r) near unity [slope = 0.99 (±0.01), r^2 = 0.999, n = 43], indicating a compound-independent response. Eight different carbonaceous materials also were analyzed by three methods, in-house by thermal-optical analysis and by two other methods used by two external laboratories. Sample materials included, DPM, coals, urban dust, and humic acid. Thermal-optical results agreed well with those reported by the two other laboratories. The variability of the TC results for the three laboratories ranged from about 1% - 7%. These findings [Birch and Cary 1996] demonstrate that carbon in a sample is accurately quantified irrespective of compound or sample type.

In sampling DPM, different samplers gave comparable EC results because particles from combustion sources are generally less than one µm (diameter). As such, they are evenly deposited on the filter and collected with high efficiency (near 100%). In the method evaluation, different sampler types (open-face 25-mm and 37-mm cassettes,

298 personal cascade impactors, and four prototype impactors) were used to collect diesel exhaust aerosol at an express mail facility. The relative standard deviation (RSD) for the mean EC concentration was 5.6% [Birch and Cary 1996]. Based on the 95% confidence limit (19%; 13 degrees of freedom, *n* = 14) on the accuracy, the NIOSH accuracy criterion [Kennedy et al. 1995] was fulfilled. Variability for the OC results was higher (RSD = 12.3%), which is to be expected when different samplers are used to collect aerosols that contain semi-volatile (and volatile) components because these may have a filter face velocity dependence. The method precision (RSD) for triplicate analyses (1.5 cm^2 filter portions) of a 37-mm quartz-fiber filter sample of DPM was normally better than 5%, and often 2% or less [NIOSH 1994].

In the method evaluation, the limit of detection (LOD) was estimated two ways: 1) through analysis of low-level calibration standards [Birch and Cary 1996; NIOSH 1994], and 2) through analysis of pre-cleaned media blanks. In the first approach, OC standard solutions (sucrose and ethylenediaminetetraacetic acid [EDTA]) covering a range from 0.23 to 2.82 µg C (or from 0.15 to 1.83 µg C per cm^2 of filter) were analyzed. An aliquot (usually 10 µL) of the standard was applied to one end of a 1.5-cm^2 rectangular filter portion that was pre-cleaned in the sample oven just prior to application of the aliquot. The filter portion was pre-cleaned to remove any OC contamination, which can greatly increase the EC LOD when TC results are used for its estimation. After cleaning the filter portion, metal tweezers are used to remove the quartz spatula that holds the portion from the sample oven. External to the oven, the spatula is held in place by a metal bracket such that the standard can be applied without removing the filter portion from the spatula. This avoids potential contamination due to handling.

Results of linear regression of the low-level calibration data were used to calculate the LOD as 3 $\sigma y/m$, where σy is the standard error of the regression and *m* is the slope of the regression line. TC results were used rather than OC because the pyrolysis correction may not account for all of the char formed during analysis of the standard (due to low sample loading and/or the position of the aliquot in the laser). If not, a small amount of the OC will be reported as EC, introducing variability in the OC results and

increasing the LOD. The LOD estimated through the linear regression results was 0.24 µg C per filter portion, or 0.15 µg/cm^2.

A simpler approach for LOD determination is through analysis of media blanks. In the method evaluation, TC results for pre-cleaned, 1.5-cm^2 portions of the filter media were used to calculate the LOD estimate. The mean (*n* = 40) TC blank was 0.03 ±0.1 µg TC. Thus, the LOD estimated as three times the standard deviation for pre-cleaned media blanks (3 σ blank) was about 0.3 µg C. This result agrees well with the value (0.24 µg C) estimated through analysis of the standard solutions. Considering a 960-L air sample collected on a 37-mm filter and a 1.5-cm^2 sample portion, this LOD translates to an air concentration of about 2 µg/m^3 ([0.3 µg TC/1.5 cm^2][8.5 cm^2]/0.960 m^3 = 1.78 µg/m^3).

Improving the EC LOD

As with all analytical methods, the LOD is a varying number. The EC LOD that is reported for NIOSH Method 5040 (about 2 µg/m^3 or an LOQ of around 7 µg/m^3) is an upper estimate. It was based on analysis of pre-cleaned media blanks from different filter lots, over a six month period, and by different analysts at two different laboratories. Further, variability for the TC results was used to estimate the LOD rather than variability for the EC results. The combined factors gave a conservative (high) estimate of the EC LOD. Lower, values are expected, depending on the individual instrument and means by which the LOD is calculated.

As an alternative to use of TC results for pre-cleaned filter media, which requires baking filters at 500 °C or higher for an hours or more, EC blank results for as received filters can be used to estimate the EC LOD. Elemental carbon on filter media is negligible and reduced very little by pre-cleaning the media. If EC is of primary interest and the level of OC contamination is acceptable (with respect to the OC and TC LOD), as received filters can be used to determine the media blank. However, variability of the OC-EC split (due to lack of sample) must be controlled through manual adjustment of the split to the beginning of the second temperature step in the oxidative mode of the analysis. Recent (2010) estimates of the EC LODs and limits of quantitation (LOQs) determined with 25-

mm and 37-mm quartz filter media from a given lot, and with manual splits assigned, are reported in the following table (units are µg EC/cm^2):

25-mm filter	EC (n=10)	37-mm filter	EC (n=6)
Mean	0.063	Mean	0.033
Standard Deviation	0.030	Standard Deviation	0.028
LOD	0.09	LOD	0.08
LOQ	0.30	LOQ	0.28

Because there are many possible OC sources, EC is a better indicator of DPM exposure. Nevertheless, high particulate OC concentrations indicate an OC source and these data can be useful for general industrial hygiene purposes if care is taken to correct for OC media contamination. Unlike EC, OC contamination (e.g., through contact with a contaminated surface or vapor adsorption) of the quartz filter media is common. Consequently, the OC (and TC) LOD is higher than for EC, and the OC (and TC) results may have significant positive bias. Bias is especially apparent when the particulate OC air concentrations and sampled air volumes are low.

To obtain a more accurate estimate of the particulate OC air concentration, an OC blank correction should be applied. Blank correction can be accomplished by subtracting the OC media blank or by a tandem filter correction (see Organic Carbon Sampling Artifacts section), with the latter generally being more accurate. Mean OC blanks, LODs, and LOQs for 25-mm and 37-mm quartz filter media are reported in the following table (units are µg OC/cm^2):

25-mm filter	OC (n=10)	37-mm filter	OC (n=6)
Mean	1.41	Mean	1.94
Standard	0.413	Standard Deviation	0.281

Deviation			
LOD	1.24	LOD	0.843
LOQ	4.13	LOQ	2.81

Two additional sets (*n* = 5 for each) of as received, 37-mm filter media were analyzed (in 01/2010 and 04/2010) and results are comparable to those listed in the tables above. Results for the two sets, including TC results for one, are given in the following table (units are µg C/cm^2):

37-mm filter	OC	EC	OC	EC	TC
Mean	1.31	0.03	1.44	0.03	1.52
Standard Deviation	0.16	0.016	0.30	0.024	0.32
LOD	0.49	0.05	0.91	0.07	0.95
LOQ	1.64	0.16	3.04	0.24	3.18

As stated above, the LOD (and LOQ) depends on the air volume, filter size, sample portion analyzed (usually 1.5 cm^2) and media blank variability. If 0.02 µg EC/cm^2 is taken as a typical standard deviation for as received media blanks (determined with manual OC-EC split adjustments), the LOD and LOQ for different air volumes, 25-mm and 37-mm filters, and a 1.5 cm^2 filter portion would be as listed in the table below.

Results for a standard deviation that was double this value (i.e., 0.04 µg EC/cm^2) also are reported as worst case estimates:

SD blank (µg EC/cm^2)	Limit	EC limit (µg/cm^2)	EC LODs and LOQs for example sampling parameters (µg EC/m^3)					
			3 m^3 air		1 m^3 air		0.5 m^3 air	
			37-mm filter	25-mm filter	37-mm filter	25-mm filter	37mm filter	25-mm filter
0.02	LOD	0.06	0.17	0.07	0.51	0.21	1.02	0.42
	LOQ	0.20	0.57	0.23	1.70	0.69	3.40	1.38
0.04	LOD	0.12	0.34	0.14	1.02	0.42	2.04	0.83
	LOQ	0.40	1.13	0.46	3.40	1.38	6.80	2.77

Example of sampling periods and flow rates (lpm = liters per minute) required for collection of recommended air volumes (green area in table below) are listed in the following table:

	Air volume (m^3) over indicated sampling period (hours)			
Flow rate (lpm)	1	2	4	8
2	120	240	480	960
4	240	480	960	1920
6	360	720	1440	2880
7[a]	420	840	1680	3360

[a] Highest flow rate tested at NIOSH laboratory. Tested with Leland Legacy pump, 8-hour sampling period and 25-mm quartz-fiber filter.

Inter-laboratory Comparisons

When results of the initial method evaluation were published [Birch and Cary 1996], an inter-laboratory comparison was not possible because the thermal-optical instrument was available in only one laboratory. After additional laboratories acquired thermal-optical instruments, a round robin comparison [Birch 1998] was conducted. Matched sets of filter samples containing different types of complex carbonaceous aerosols were distributed to eleven laboratories. Six of the eleven analyzed the samples according to NIOSH 5040, while five used purely thermal (i.e., no char correction) methods. Good inter-laboratory agreement was obtained among the six laboratories that used NIOSH 5040. In the analysis of samples containing DPM, the variability (RSD) for the EC results ranged from 6% to 9%. Only low EC fractions were found in wood and cigarette smoke. Thus, these materials pose minimal interference in the analysis of EC. In addition, only minor amounts of EC were found in two OC standards that char: about 1% and 0.1% for sucrose and the disodium salt of ethylenediaminetetraacetic acid (EDTA), respectively. Two aqueous solutions of OC standards were included in the comparison as a check on the validity of the char correction and accuracy of the TC results. Variability (RSD) of the TC results for the two standard solutions and five filter samples ranged from 3% to 6%.

A second inter-laboratory comparison study on NIOSH 5040 has been reported [Schauer et al. 2001]. Seven environmental aerosol samples were analyzed in duplicate by eight laboratories. Four samples were collected in U.S. cities, and three were collected in Asia. Inter-laboratory variability for the EC results ranged from 6% to 21% for six samples having EC loadings from 0.7 to 8.4 µg/cm^2. Four of the six had low EC loadings (0.7 µg/cm^2 to 1.4 µg/cm^2). The variability for the OC results ranged from 4% to 13% (OC loadings ranged from about 1 to 25 µg/cm^2). Results for TC were not reported, but the variability reported for the OC results should be representative of that for TC because the samples were mostly OC (75% to 92%).

Carbonates

Carbonate in a sample is indicated by a narrow peak during the fourth temperature step in helium [Birch 2004a]. Its presence is verified by exposing a second portion of the filter to hydrogen chloride (HCl) vapor prior to analysis. When the acidified portion is analyzed, a diminished (or absent) peak during the fourth temperature step is indicative of carbonate in the original sample [Birch 2004a]. Environmental samples typically contain little (if any) carbonate, but carbonate (e.g., in limestone, trona, concrete) levels in some occupational samples can be quite high. In such cases, it is important to ensure that all of the carbonate is removed during the first stage of the analysis. If it is not completely removed (because of high loading) the sample should be acidified.

Organic Carbon Sampling Artifacts

Problems commonly referred to as 'sampling artifacts,' have been reported when collecting particulate OC on quartz fiber filters. These artifacts do not affect the EC results, but cause positive or negative bias in the measurement of particulate OC (and TC). Eatough et al. [1995, 1996] observed loss of semi-volatile OC from particles during sampling, referred to as the 'negative' or evaporation artifact. This artifact causes a negative bias in the particulate OC (and TC) concentration because OC initially collected as condensed matter is subsequently lost through evaporation from the filter during sampling. Conversely, several studies have demonstrated a 'positive' or adsorption artifact due to filter adsorption of gas phase OC. A quartz-fiber filter collects airborne particulate matter and allows gases and vapors to pass through, but some adsorption of gas phase (and vapor) OC occurs, resulting in overestimation of the true airborne particulate OC concentration [Turpin et al. 2000; McDow and Huntzicker 1990; Turpin and Huntzicker 1994; Olson and Norris 2005; Kirchstetter et al. 2001; Mader et al. 2003; Subramanian et al. 2004; Mader et al. 2001; Noll and Birch 2008; Schauer et al. 1999].

Most of the studies on sampling artifacts apply to environmental air sampling. Occupational sampling methods and conditions are generally much different than

environmental. Environmental samples are usually collected at much higher face velocities: 20-80 cm/s as opposed to 3-4 cm/s for occupational samples. In addition, the concentrations of carbon are much lower in environmental air than in most occupational settings [Fruin et al. 2004; Sheesley et al. 2008], and the types of aerosols sampled are different (e.g., aged aerosol from multiple environmental sources, as opposed to aerosols close to source). These differences are important because OC sampling artifacts depend upon conditions such as filter face velocity, air contaminants present, sampling time, and filter media. Given the much lower filter face velocities typical of occupational sampling, adsorption (i.e., positive artifact) is expected over evaporation for occupational samples. Turpin and Huntzicker [1994], Kirchstetter et al. [2001], Noll and Birch [2008], and Schauer et al. [1999] have reported adsorption as the dominant artifact.

To correct for the positive adsorption artifact, tandem quartz filters have been applied. When sampling with tandem filters, particulate matter is collected by the first filter, while both the first and second filters are exposed to and adsorb gaseous and vaporous OC. For the correction to be effective, both filters must be in equilibrium with the sampled airstream, adsorb the same amount of gas/vapor OC, and not have a significant amount of OC loss through evaporation. The OC on the second filter can then be subtracted from the OC on the first filter to account for the adsorbed OC. Several studies have found the tandem filter correction to underestimate the adsorption artifact [Turpin et al. 2000; McDow and Huntzicker 1990; Turpin and Huntzicker 1994; Olson and Norris 2005], while others have shown effective correction [Kirchstetter et al. 2001; Mader et al. 2003; Subramanian et al. 2004; Mader et al. 2001; Noll and Birch 2008]

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Air samplers containing a Teflon® and quartz filter also have been used for correction of the positive OC artifact. In theory, the Teflon® top filter collects particulate matter with negligible OC gas/vapor adsorption, so only the quartz filter beneath it adsorbs gas and vapor OC. Studies on tandem filter corrections have shown the quartz filter beneath Teflon® to have a greater OC value than quartz beneath quartz [Turpin et al. 2000; Olson and Norris 2005]. This finding was attributed to the quartz beneath quartz not reaching equilibrium with the sampling stream and underestimating the adsorption

artifact. Others have attributed it to the evaporation artifact being more prevalent when using a Teflon® filter instead of a quartz filter and reported the quartz behind Teflon® to overestimate the adsorption artifact [Subramanian et al. 2004. Several studies have shown no difference when using either type of correction [Mader et al. 2003; Mader it al. 2001].

Noll and Birch [2008] conducted studies on OC sampling artifacts for occupational samples to test the accuracy of the tandem quartz filter correction. In practice, using two quartz filters for air sampling is preferable to the Teflon®-quartz combination because both the collection and blank filters are in the same sampler. The tandem quartz correction effectively reduced positive bias for both laboratory and field samples. Laboratory samples were collected under conditions that simulated DPM sampling in underground mines. Without correction, TC on the sample filter was 30% higher than the actual particulate TC for 50% of the samples, but was within 11% of the particulate TC after the tandem quartz fiber correction. For field samples, this correction significantly reduced positive bias due to OC adsorption artifact. Little artifact effect was found after the correction was made.

Other Applications

Method 5040 has application to other types of carbonaceous aerosols. When applied to materials such as carbon black or carbon nanofibers/nanotubes (CNF/CNT), particle deposition on a filter may be more variable because particles in these materials are much larger than DPM. Variability depends on the sampler type, and as expected, different samplers (e.g., cyclones, open- and closed-face cassettes) will give different air concentration results, depending on the particle size distribution. Diesel emissions, and combustion aerosols generally, are composed of ultrafine (< 100 nm diameters) particles. Because of the small size, DPM normally deposits evenly across the quartz-fiber filter used for sample collection. As already discussed, even deposition is required because only a portion of the filter is normally analyzed so it must be representative of the entire sample deposit.

When applying NIOSH 5040 to carbonaceous dusts, it is important to verify an even filter deposit so that an accurate air concentration (based on results for the filter portion) can be calculated. Alternatively, the entire filter can be analyzed if the deposit is uneven, but this requires analysis of multiple portions of a 37 mm filter due to the relatively small diameter (about 1 cm) of the carbon analyzer's quartz sample oven. Quality assurance procedures should include duplicate analyses of the 37 mm filter to check precision, especially if the deposit appears uneven. If a 25 mm filter is used, the entire filter can be analyzed, which both improves the LOD and obviates the need for an even deposit, but a repeat (or other chemical analysis0 of the sample is not possible if the entire filter is analyzed. Additional details on the evaluation and use of NIOSH 5040 are provided elsewhere [Birch and Cary 1996; Birch 1998; Birch et al. 1999; Birch 2002; Birch 2003; Birch 2004a].

Among other measurements, NIOSH 5040 was applied for area monitoring at a laboratory facility that processes CNF in the production of polymer composites [Methner et al. 2007]. Carbon nanofibers and CNT have negligible (if any) OC content, making EC a good indicator of these materials. Survey results were reported in terms of TC, which is subject to OC interferences, but the OC results were blank corrected to minimize the positive sampling artifact. Further, based on the thermal profiles for the air samples and the bulk materials (CNF and composite product), TC (blank corrected) was a good measure of the CNF air concentration except in an area where a wet saw was operating. In that area, TC was a measure of the composite aerosol released during the sawing operation.

Extensive air monitoring was conducted at second facility that manufactures and processes CNFs [Evans et al. 2010]. The relative percent difference (RPD) and RSD (%) for repeat analyses of 12 samples collected in different areas of the facility are listed in Table 1. Total, thoracic, and respirable dust samples are included. Total dust was collected with 37-mm cassettes, while cyclones were used to collect thoracic and respirable dust. The RPD was determined by analyzing either two punches from the same filter (duplicates) or one punch from two different filters (paired samplers); the

RSD was determined by analyzing one filter in triplicate. The precision for the EC results ranged from about 3% to 14% except for one respirable sample, where the RPD was about 22%. Higher variability for the latter may relate to the filter punches being from different samplers, but precision in two other instances, where punches were from different samplers, had RPDs of about 8% and 13%, comparable to results for multiple punches from the same filter.

Table 1. NIOSH 5040 precision for air samples collected in different areas of a CNF manufacturing facility with 37-mm cassettes (total dust) and cyclone samplers (thoracic and respirable dust). OC, EC, TC are reported as air concentrations (µg C/m^3).

Sample	OC[a]	RPD or RSD[b] (%)	EC[c]	RPD or RSD (%)	TC	RPD or RSD (%)	Comments
Respirable	16.42	0.97	[1.87][d]	13.37	18.28	2.19	paired[e]
Respirable	22.19	8.25	3.41	22.29	25.66	10.60	paired
Total	27.17	13.40	21.52	12.04	48.69	12.80	duplicate[f]
Respirable	60.87	0.74	79.59	12.14	140.31	6.36	duplicate
Respirable	25.47	4.46	20.72	8.48	46.09	6.28	duplicate
Total	12.42	6.84	4.14	4.59	16.60	4.88	triplicate[g]
Respirable	19.89	3.22	3.05	4.59	22.93	2.22	triplicate
Total	15.11	1.29	9.89	9.37	25.01	3.63	triplicate
Total	17.80	9.72	11.07	7.97	28.88	9.15	paired
Thoracic	27.16	10.80	11.23	6.79	38.46	6.68	triplicate
Respirable	22.81	2.50	23.67	13.86	46.48	8.26	duplicate
Respirable	18.64	6.77	8.44	3.15	27.14	5.63	duplicate

[a]OC = organic carbon. [b]RPD is relative percent difference. RSD is relative standard deviation.
[c]EC = elemental carbon. [d]Result in brackets lies between method LOD and LOQ.
[e]Results for two identical, paired samplers. [f]Duplicate analysis of same filter. [g]Triplicate analysis of same filter.