Application and testing of risk screening tools for nanomaterial risk analysis

Grieger, Khara Deanne; Bossa, Nathan; Levis, James W.; von Borries, Kerstin Johanna Felicitas; Strader, Phillip; Cuchiara, Maude; Hendren, Christine Ogilvie; Hansen, Steffen Foss; Jones, Jacob L.

Published in:
Environmental Science: Nano

Link to article, DOI:
10.1039/c8en00518d

Publication date:
2018

Document Version
Peer reviewed version

The Difficulties in Establishing an Occupational Exposure Limit for Carbon Nanotubes

M. Ellenbecker, S-J. Tsai, M. Jacobs, M. Riediker, T. Peters, S. Liou, A. Avila, S. Foss Hansen

M. Ellenbecker
Toxics Use Reduction Institute
University of Massachusetts Lowell
Lowell, MA 01854, USA

S-J. Tsai
Department of Environmental and Radiological Health Science
Colorado State University
Fort Collins, CO 80523-1681, USA

M. Jacobs
Lowell Center for Sustainable Production
University of Massachusetts Lowell
Lowell, MA 01854, USA

M. Riediker
Institute for Work and Health
Lausanne CH-1066, Switzerland

T. Peters
Department of Occupational and Environmental Health
The University of Iowa
Iowa City, IA 52242, USA

S. Liou
National Institute of Environmental Health Sciences
Zhunan Town, 35053, Taiwan

A. Avila:
Dept. of Electrical and Electronic Engineering and Centro de Microelectrónica (CMUA),
Universidad de los Andes, Bogotá 11001, Colombia

S. Foss Hansen
Department of Environmental Engineering
Technical University of Denmark
Lyngby, Denmark

Corresponding author:

M. Ellenbecker
ellenbec@turi.org
Phone: 1.978.934.3272
Fax: 1.978.934.3050

Abstract

Concern over the health effects from the inhalation of carbon nanotubes (CNTs) has been building for some time, and adverse health effects found in animal studies include acute and chronic respiratory damage, cardiac inflammation, and cancer including mesothelioma, heretofore only associated with asbestos exposure. The strong animal evidence of toxicity requires that the occupational hygiene community develop strategies for reducing or eliminating worker exposures to CNTs; part of this strategy involves the setting of occupational exposure limits (OELs) for CNTs. A number of government agencies and private entities have established OELs for CNTs; some are mass-based, while others rely on number concentration. We review these various proposed standards and discuss the pros and cons of each approach. We recommend that specific action be taken, including intensified outreach to employers and employees concerning the potential adverse health effects from CNT inhalation, the development of more nuanced OELs that reflect the complex nature of CNT exposure, a broader discussion of these issues among all interested parties, and further research into important unanswered questions including optimum methods to evaluate CNT exposures. We conclude that current animal toxicity evidence suggests that strong action needs to be taken to minimize exposures to CNTs, and that any CNT OEL should be consistent with the need to minimize exposures.

Introduction

Concern over the health effects from the inhalation of carbon nanotubes (CNTs) has been building for some time. A review of articles published over the past dozen years (Boxall et al.

2007; Donaldson et al. 2006; Kisin et al. 2007; Kisin et al. 2011; Lam et al. 2006; Legramante et al. 2012; Li et al. 2007; Ma-Hock et al. 2009; Muller et al. 2005; Shvedova et al. 2003; Shvedova et al. 2008a; Shvedova et al. 2005; Shvedova et al. 2008b; Shvedova et al. 2008c; Simeonova 2009; Warheit et al. 2004) outlines the growing concerns regarding the toxicity of CNTs. Recent review papers (Ema et al. 2016; Gao et al. 2016; Kuempel et al. 2016; Ong et al. 2016; Pacurari et al. 2016; Siegrist et al. 2014; Vietti et al. 2016) provide an excellent overview of the current knowledge regarding adverse health effects of single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). The Organization for Economic Cooperation and Development (OECD) has recently published comprehensive summaries of the environmental health and safety aspects of both MWCNTs (OECD 2016a) and SWCNTs (OECD 2016b). Rodent studies have found an acute inflammatory response, granulomas, fibrosis, and decreased rates of respiration and bacterial clearance from the lungs. Importantly, the National Institute for Occupational Safety and Health (NIOSH) (NIOSH 2013) concluded that "…in animal studies where CNTs were compared with other known fibrogenic materials (e.g., silica, asbestos, ultrafine carbon black), the CNTs were of similar or greater potency, and the effects, including fibrosis, developed soon after exposure and persisted." Adverse impacts on other organ systems, including cardiac inflammation, have also been found (NIOSH 2013). Such a wide range of acute and chronic health effects associated with CNTs, particularly the strong fibrogenic potential, are reason enough for concern – but even more serious concerns have arisen. Almost twenty years ago, the morphological similarity between CNTs and other fibrous materials, such as asbestos, raised concerns as to whether exposure to CNTs could cause lung cancer and/or mesothelioma (Service 1998). Research followed, and two groups observed asbestos-like effects in short-term bioassays when MWCNTs were injected intraperitoneally into mice (Poland et al.

2008; Takagi et al. 2008); subsequently, Ryman-Rasmussen *et al.* (Ryman-Rasmussen et al. 2009)found that inhaled MWCNTs reached the subpleura of mice and Mercer *et al.* (Mercer et al. 2010) found that they penetrated the intrapleural space. Additional research has for the most part confirmed the results of the first studies (Muller et al. 2009; Nagai et al. 2011; Rittinghausen et al. 2014; Schinwald et al. 2012), while others were negative (Muller et al. 2009).

Recently, the International Agency for Research on Cancer (IARC) reviewed the available toxicological studies and classified certain MWCNTs as a Group 2B carcinogen, where Group 2B is defined as "possibly carcinogenic to humans" (Grosse et al. 2014; IARC 2017; Kuempel et al. 2016). IARC based its classification on the Poland and Takagi rodent studies, which used a particular MWCNT designated "MWCNT-7," and its classification applies only to this particular product. Specifically, IARC found that "inhalation of MWCNT-7 promoted bronchioloalveolar adenoma and carcinoma in male mice" and "MWCNT-7 caused peritoneal mesotheliomas in male and female rats in one intraperitoneal injection study and one intrascrotal injection study, and in male p53+/- mice in two intraperitoneal injection studies"(Grosse et al. 2014). Although rodents were exposed by routes other than inhalation, IARC referenced Mercer *et al*. (Mercer et al. 2010) to conclude that "mechanistic and other data in rodents provided evidence of trans location of three types of MWCNTs (including MWCNT-7) to the pleura." The Rittinghausen paper (Rittinghausen et al. 2014) was published after the IARC review occurred, and found that four different MWCNTs induced mesothelioma in 40-98% of the rats tested.

All evidence for adverse health effects is based on animal toxicity studies; no case reports or epidemiological studies of CNT-specifically exposed workers have been published. Oberdörster, et al. (Oberdörster et al. 2015) discuss in detail the difficulties in conducting a proper animal inhalation study for CNTs, including e.g. the use of different delivery techniques (instillation,

aspiration, inhalation), high doses, high dose rates, pretreatment with dispersants, poor distribution throughout the respiratory tract, etc. These difficulties and differences between studies make it very difficult to translate results of rodent studies to levels of exposure likely to cause adverse health effects in humans. However, the animal studies, taken together, seem to indicate that at least some MWCNTs cause the same three major diseases associated with asbestos use (pulmonary fibrosis, lung cancer and mesothelioma) and in fact may be a more potent cause of these very serious diseases. The history of asbestos exposure and disease is well-known, and leads to the obvious questions as to whether the occupational and environmental health community can take proper action to prevent another similar pattern of exposure and disease development. Such questions are ones of broad public health policy, with implications well beyond occupational hygiene. We believe that the occupational and environmental health community in particular must act proactively to ensure that workers and members of the public are not needlessly exposed to what may in the future be confirmed as a human carcinogen. The strong animal evidence of toxicity requires that the occupational hygiene community develop strategies for reducing or eliminating worker exposures to CNTs.

This commentary focuses on the issue of setting appropriate occupational exposure levels (OELs) for CNTs, although many issues must be addressed, including exposure assessment methodologies and effective exposure control strategies. We first describe the OELs suggested by government agencies and companies; at this time there are no regulatory OELs specific to CNTs. We then discuss important issues that must be addressed in the setting of an OEL for CNTs, including the more fundamental question about the appropriateness of OELs for suspected carcinogens. We close with some recommendations for actions we believe should be taken in the near future to address this important issue.

137

138 **Recommended OELs**

139 In response to the adverse health effects found in animal studies, several governmental
140 agencies, and one private company, have published occupational exposure limits for CNTs.
141 These are briefly reviewed here.

142 The British Standards Institute (BSI) in 2007 recommended a "benchmark" CNT OEL of
143 0.01 fibers/cm^3 (f/cm^3), as measured by scanning or transmission electron microscopy (BSI
144 2007). This level is equivalent to the most rigorous exposure limit in Britain for asbestos, *i.e.*, the
145 highest concentration that can be present inside a space after asbestos removal activities (also
146 called the clearance limit, this is the same limit as used by the US EPA for this activity).

147 The German company Bayer Schering Pharmaceuticals studied the toxicity of their
148 MWCNTs, called Baytubes. They concluded that exposure is unlikely to lead to mesothelioma or
149 other chronic conditions because Baytubes are flexible, leading to the formation of relatively
150 large assemblages, or "bird's nests" of tubes. They set a company OEL for Baytubes of 50
151 μg/m^3 , based on measured acute toxicity in rats (Pauluhn 2010). Pauluhn stated that their
152 measurements of Baytube mass concentration were made "utilizing cobalt [a catalyst used in
153 Baytube manufacturing] as a tracer (in order to distinguish carbonaceous background dust from
154 Baytubes)" but no more details of the measurement method were provided.

155 The Japanese National Institute of Advanced Industrial Science and Technology (AIST)
156 derived OELs of 30 μg/m^3 for SWCNTs and 80 μg/m^3 for MWCNTs (Nakanishi 2011), based on
157 studies supported by the New Energy and Industrial Technology Development Organization

158 (NEDO) of Japan. These limits were based on no observed effect levels (NOELs) calculated for
159 non-carcinogenic effects.

160 The Swiss Accident Insurance Funds (SUVA) addressed carbon nanotubes and fibers in the
161 Swiss 2011 occupational exposure limit list (SUVA 2011). The document highlighted the
162 structural similarities of CNTs and CNFs to other fibers such as asbestos and noted that these
163 materials lead to inflammation. The document specifically mentioned that studies done with long
164 rigid MWCNTs suggest that they may be carcinogenic; consequently, they recommended an
165 exposure limit of 0.01 f/cm^3 for CNTs and CNFs. This limit corresponds to their threshold value
166 for asbestos fibers and remains in the latest (2015) edition of the occupational exposure limit list.

167 The German Institute for Occupational Safety and Health (IFA) developed "benchmark"
168 levels for evaluating engineered nanoparticle (ENP) exposures, based on what IFA considers to
169 be likely predictors of ENP toxicity, i.e., size, shape, density and biopersistence. Four groups are
170 defined, each with a "nano reference value (NRV)." Group 1 consists of "rigid, biopersistent
171 nanofibers for which effects similar to those of asbestos are not excluded" (e.g., CNTs) with a
172 NRV of 0.01 f/cm^3 (the same as the BSI recommendation for CNTs and asbestos) (van
173 Broekhuizen and Dorbeck-Jung 2013). It is clear that the NRVs are meant to be differentiated
174 from actual health-based OELs, and are to be used as interim exposure guidelines until OELs can
175 be developed (van Broekhuizen et al. 2012).

176 After much discussion of an earlier draft, in 2013 NIOSH published Current Intelligence
177 Bulletin (CIB) with a recommended exposure limit (REL) of 1 μg/m^3 of elemental carbon (EC)
178 (NIOSH 2013). This limit is based on the limit of quantitation (LOQ) of Method 5040, titled
179 "Diesel Particulate Matter (as Elemental Carbon)" (NIOSH 2003).They calculate that the LOQ
180 "can be obtained for an 8-hr respirable sample collected on a 25-mm filter at a flow rate of 4

liters per minute (lpm)." Regarding health effects, for a 45-year lifetime exposure at the REL, NIOSH developed "maximum likelihood estimates" of 2.4 – 33% for "minimal lung effects" and 0.23 – 10% for "slight or mild lung effects" as. The CIB concluded that "NIOSH does not consider a 10% estimated excess risk over a working lifetime to be acceptable for these early-stage lung effects, and the REL is set at the optimal limit of quantification (LOQ) of the analytical method carbon (NIOSH method 5040)."

Carcinogenic potential was not considered in setting the REL. "NIOSH has determined that the best data to use for a quantitative risk assessment and as basis for a recommended exposure limit (REL) are the nonmalignant pulmonary data from the CNT animal studies. At present, data on cancer and cardiovascular effects are not adequate for a quantitative risk assessment of inhalation exposure" (NIOSH 2013).

To summarize, various entities have recommended both mass-based and number-based OELs for CNTs, as shown in Table 1. The number-based recommendations all are consistent with the strictest asbestos OEL of 0.01 f/cm^3, whereas the mass-based recommendations range from 1 – 80 μg/m^3.

Advantages and Disadvantages of a Mass-Based OEL for CNTs

AIST, Bayer and NIOSH developed mass-based CNT OELs for some very good reasons. One advantage, as discussed above, is that the use of mass concentration correlates well with non-carcinogenic end-points in animal toxicity studies. The primary benefit of this approach, however, is that it uses classic occupational hygiene measurement methods and metrics. Any OEL loses most of its utility if there are no methods to measure worker exposure for comparison

to the standard. For example, the NIOSH REL requires the use of readily-available air sampling equipment and a validated sample analysis method that can be performed by many laboratories at as reasonable price. Thus, any reasonably-proficient field occupational hygienist can collect a valid sample and compare it to the REL. This advantage makes a compelling reason for using this approach.

There are, however, several concerns with using a mass-based OEL for CNTs. First, the risks of developing the most serious adverse health effects, *i.e.,* fibrosis, lung cancer and mesothelioma, are a function not of the *mass* of CNTs inhaled but on the *number* of appropriately-sized fibers inhaled and subsequently depositing in alveoli. Since the mass of any individual CNT can vary greatly, a given mass concentration can have a widely ranging number concentration, so that a mass-based OEL does not correlate well with the property of interest, number. Second, an air sample is likely to collect both CNTs and other particles, but the available analytical methods cannot directly measure the mass of CNTs collected. Available methods rely on a surrogate of CNT mass, such as cobalt for Baytubes and elemental carbon for NIOSH. While it is true that CNTs consist largely of elemental carbon, there may other sources of elemental carbon in the workplace, such as carbon soot formed by incomplete tube formation in a CNT furnace, or diesel exhaust from fork lift trucks in a factory incorporating CNTs into a product.

A third concern with a mass-based OEL is that the actual values proposed correspond to number concentrations that can be much higher than asbestos OELs because they are based either on acute health effects for a specific tested CNT (the AIST OEL of 80 $\mu g/m^3$) or on available analytical methods (the NIOSH REL of 1 $\mu g/m^3$). The issue was discussed by Schulte, et al. (Schulte et al. 2012), who compare fiber number concentrations for fibers of different

dimensions to a mass concentration of 7 μg/m^3 (this was the original proposed REL of NIOSH). Adjusting their conversions to 1 μg/m^3, this corresponds to 0.01 fibers/cm^3 for a very large fiber (2,110 nm diameter x 10,000 nm length) and 300,000 fibers/cm^3 for a very small fiber (2 nm x 500 nm). These fiber concentrations range from lower than the asbestos PEL of 0.1 f/cm^3 to much higher than the PEL (OSHA 1994).

Advantages and Disadvantages of a Number-Based OEL for CNTs

The use of a fiber number-based OEL also presents distinct advantages and disadvantages. The primary advantage is that the risk of developing the serious chronic diseases that have been associated with CNT exposure in animal studies – fibrosis, lung cancer, and mesothelioma – are all a function of the number of fibers deposited in the alveolar region of the lung, not the mass. It is for this reason that all asbestos OELs are given in f/cm^3. The primary disadvantage of a number-based OEL is that it is difficult and costly to obtain exposure measurements. Breathing-zone asbestos concentrations are measured by passing the sampled air through a cellulose ester membrane filter and examining the filter with a phase contrast optical microscope. Unfortunately, CNTs are too small to be seen by an optical microscope, and electron microscopy must be used, increasing the cost of analysis by at least an order of magnitude. Direct-reading particle counters can also be used, but they are expensive and count all particles, not just fibers. We will return to this measurement conundrum in the Recommendations section.

Possible Variations between Different CNT Types

A one-size-fits-all OEL is unlikely to adequately protect workers because the literature suggests that there may be important differences in toxicological response among types of CNTs. In various studies, single wall carbon nanotubes (SWCNTs) have not been found to cause mesothelioma (Kuempel et al. 2016). In addition, thin ($d < 15$ nm) and short [$L < 1$ μm according to Muller et al. (Muller et al. 2009) and $L < 5$ μm according to Schinwald et al. (Schinwald et al. 2012) MWCNTs do not cause mesothelioma. SWCNTs and thin MWCNTs, when examined microscopically, tend to curl and form bundles which are not fiber-shaped. Presumably, this shape enhances their clearance from the pulmonary region by phagocytosis. Likewise, very short MWCNTs may be cleared effectively by macrophages (Rittinghausen et al. 2014). As an added complication, Nagai et al. (Nagai et al. 2011) found that very thick MWCNTs ($d > 150$ nm) were less carcinogenic than thinner ones; however, there is evidence that such large-diameter tubes are not important commercially.

The absence of mesothelioma initiation when short tubes are administered to test animals suggests the possibility of treating pristine tubes to shorten them. Ali-Boucetta et al. (Ali-Boucetta et al. 2013) used two different reactions to functionalize pristine long MWCNTs and found that one reaction (functionalization with TEG chains using the 1,3-dipolar cycloaddition reaction) led to a reduction of the effective length of the MWCNTs, while a second reaction (functionalization with octyl chains following the Billups reaction) did not. These results suggest that functionalization needs much further research, and in any case must be used with great care. For example, procedures applied in the laboratory that shorten 100% of the tubes may have lower efficiency when applied at an industrial scale. In addition, many industrial processes may require the use of longer tubes, eliminating this option from consideration.

Another approach that may be effective in certain applications where the MWCNTs are dispersed in water is to coat them with a surfactant. Wang et al. (Wang et al. 2012) found that dispersing MWCNTs in Pluronic F 108, a difunctional block copolymer surfactant, reduced fibrogenic response by reducing damage to the lysosomal membrane.

In any case, MWCNTs subject to any such treatments could never be considered completely safe, since the possible exposure of the workers manufacturing the pristine tubes and functionalizing them would have to be considered. For example, to coat CNTs with a surfactant, the dry tubes would have to be dispersed into the water and surfactant, a potentially hazardous operation.

Agglomerates vs. Individual Fibers

Another significant complication that is not addressed by using a mass-based OEL is that airborne CNTs may exist as individual fibers or as agglomerates or bundles of fibers. The state of agglomeration can influence both the respiratory deposition pattern of the inhaled fibers and the toxicological response. Researchers have found varied changes in toxicity when CNTs agglomerate. For example, Gao et al. (Gao et al. 2016) found that "it clearly appears that aggregation of SWCNTs should be avoided and that nanotube individualization is a key parameter to minimize cellular toxicity." Wick et al. (Wick et al. 2007) found that agglomerated MWCNTs were more toxic than well-dispersed ones. On the other hand, end effects that depend on the fibrotic nature of CNTs (fibrosis and mesothelioma) should presumably be ameliorated by the formation of non-fiber-shaped agglomerates (Kuempel et al. 2016). Song et al. (Song et al. 2016) summarized the current muddled state of the research on this topic thus: "more efforts should be paid to study the biological effects of agglomeration." In any case, a significant

shortcoming of mass-based OELs is that the state of agglomeration in the sample would not be known.

From an occupational hygiene viewpoint, agglomeration complicates the use of number-based OELs. Counting schemes need to be developed to address this issue; it is likely that an approach similar to that used for high aspect ratio particles such as bundles of asbestos fibers, where clearly-identified fiber "ends" in fiber bundles are counted, may be needed. NIOSH in 2016 published a draft analytical method titled "Analysis of Carbon Nanotubes and Nanofibers on Mixed Cellulose Ester Filters by Transmission Electron Microscopy" (Birch et al. 2016), which was a modification to NIOSH NMAM 7402, asbestos by TEM (NIOSH 1994). It is an initial attempt to develop an approach in the United States.

Actions by Other Government Agencies

The U.S. Environmental Protection Agency (EPA) regulates CNTs under the Toxics Substance Control Act (TSCA). CNTs were designated as a material requiring a premanufacture notification (PMN), and, as an example, in September, 2017 the EPA issued a significant new use rule (SNUR) for a specific CNTs used in filtration media (EPA 2017). The company-specific SNUR requires the use of protective clothing and NIOSH-approved respirators where there is as potential for exposure, processing and use of only those quantities specified in the consent order, processing only as a aqueous slurry, wet form, or "contained" dry form, prohibits release of CNTs to surface waters, and requires disposal to be done only by landfill or incineration. The SNUR's restrictions on manufacture, processing, distribution in commerce, and disposal will remain in effect until the results of recommended testing is completed (2-year

inhalation bioassay; daphnid chronic; and algal toxicity). Such actions by EPA, done in consultation with NIOSH and OSHA, serve as an interim approach to worker exposure while awaiting the results of recommended toxicity testing.

The European Union′s law regarding Registration, Evaluation, Authorization and Restriction of Chemical substances (REACH), which entered into force on June 1, 2007 (Commission of the European 2007), may also offer some protection to workers potentially exposed to CNTs. TSCA and REACH differ greatly in their approaches to regulating chemical health and safety, and a detailed comparison of the two approaches is beyond the scope of this paper. Readers interested in such a comparison are referred to Chapter 11 of the textbook by Ellenbecker and Tsai (Ellenbecker and Tsai 2015). Briefly, it is fair to say that REACH does not provide the detailed performance standards specified in a SNUR, but rather requires manufacturers to proactively ensure that their products are manufactured and used safely. In addition, the European Commission has promulgated a recommended "code of conduct for responsible nanosciences and nanotechnologies (N&N) research" (EC 2008). Key elements of the code of conduct include:

> N&N research activities should be safe, ethical and contribute to sustainable development serving the sustainability objectives of the Community as well as contributing to the United Nations' Millennium Development Goals. They should not harm or create a biological, physical or moral threat to people, animals, plants or the environment, at present or in the future.
>
> N&N research activities should be conducted in accordance with the precautionary principle, anticipating potential environmental, health and safety impacts of N&N outcomes and taking due precautions, proportional to the level of protection, while encouraging progress for the benefit of society and the environment.
>
> Governance of N&N research activities should be guided by the principles of openness to all stakeholders, transparency and respect for the legitimate right of

342 access to information. It should allow the participation in decision-making
343 processes of all stakeholders involved in or concerned by N&N research
344 activities.

345

346 The EC recommends:

347 That Member States encourage the voluntary adoption of the Code of Conduct by
348 relevant national and regional authorities, employers and research funding bodies,
349 researchers, and any individual or civil society organization involved or interested
350 in N&N research and endeavor to undertake the necessary steps to ensure that
351 they contribute to developing and maintaining a supportive research environment,
352 conducive to the safe, ethical and effective development of the N&N potential.

353

354 **Other Considerations**

355

356 Other important CNT occupational hygiene issues, such as exposure assessment and control,
357 depend to some extent on decisions made about appropriate OELs. Methods used to evaluate
358 exposure will differ greatly for a mass-based OEL and a number-based OEL. An advantage of
359 the mass-based OELs when compared to a number-based OEL is that personal samples can be
360 collected and analyzed using readily-available and well-understood equipment and techniques. In
361 contrast, measuring the number concentration and size distribution of nanometer-sized fibers
362 requires expensive, specialized equipment and operator skill and is limited at this time to area
363 samples, with no agreed-upon technique to be used.

364 The measurement of a very low number concentration of MWCNTs of a certain size will
365 likely require the development and validation of a new method based on transmission electron

366 microscopy. However, any CNT OEL is likely to require some form of electron microscopy in
367 order to ensure that what is being sampled and analyzed actually contains CNTs. The CIB further
368 recommends that EC and electron microscope samples be collected in parallel and that for each
369 EC sample where the concentration that exceeds the NIOSH REL, the electron microscope
370 sample should be analyzed to confirm that the EC actually came from CNTs.

371 All of the proposed OELs represent very low levels of exposure, and effective administrative
372 and engineering controls will be required to reduce exposures to acceptable levels. Research has
373 demonstrated that standard control practices, such as local exhaust ventilation and high-
374 efficiency particulate air (HEPA) filtration, when applied with care, can effectively control
375 nanoparticle exposures to minimal level (Golanski et al. 2010; Tsai et al. 2009a; Tsai et al.
376 2008b; Tsai et al. 2012), and publications on best practices are widely available (BSI 2007; DOE
377 2008; NCI 2008; NIOSH 2012; Wood 2000). Large MWCNTs will require, however, the highest
378 level of controls to reduce exposures to a concentration such as the BSI benchmark.
379 Sophisticated containment systems, such as those used by the pharmaceutical industry (Wood
380 2000), may be required.

381 The recent IARC classification for MWCNT-7 raises a more significant question about
382 establishing OELs, namely, should we even be issuing OELs for carcinogens or suspected
383 carcinogen where the evidence is clear that the "best" exposure limit is no exposure.

384 Some might argue that the "lowest possible level of exposure" approach be limited to IARC
385 1A confirmed human carcinogens and thus is overly strict for MWCNTs, an IARC 2B suspect
386 human carcinogen (Grosse et al. 2014; IARC 2017; Kuempel et al. 2016). However, the 1A
387 designation is only given to substances with sufficient positive epidemiologic evidence of an
388 association between the substance and the cancer. Effectively, every new 1A designation

represents a case of evident exposure to workers, since in every case there is evidence of toxicity before the 1A designation and, in spite of that evidence, exposures were allowed sufficient to lead to a statistically significant level of cancer. Recognizing this, the American Conference of Governmental Industrial Hygienists states that for suspected human carcinogens, "worker exposure by all routes should be carefully controlled to levels as low as possible…" (ACGIH 2012) Our goal should be that there is *never* a positive epidemiology study for MWCNTs (or any other engineered nanoparticle, for that matter).

Rather than presuming that we can control workplace exposures to CNTs, especially those with evidence of carcinogenicity, there is a need to redirect technological developments that are venturing down unsafe paths. At a minimum, each potential use of MWCNTs must first undergo a rigorous analysis of the potential benefits versus the possible risks. Two hypothetical examples may illustrate this point. The first is the incorporation of MWCNTs into tennis racquet frames. This would require the use of relatively large amounts of dry MWCNTs being mixed with the polymer in an extruder, with high potential for exposure to workers (Tsai et al. 2008a) and would offer minor benefits to society. The second example is the use of MWCNTs as advanced memory storage devices in electronics. The manufacturing process would use minimal amounts of MWCNTs suspended in water, with minimal potential for worker exposure or environmental release, while potential benefits to society are very large. The first example may fail the risk-benefit test, while the second may pass it.

Such an approach is consistent with the European Union's 2004 workplace carcinogen directive (EU 2004), which requires that employers replace the use of carcinogens with less dangerous substitutes wherever feasible. History has shown us too many public and occupational

health tragedies where society allowed the proliferation in use of suspected carcinogens by industry while scientists waited for evidence in humans to mount. Is the risk worth the wait?

Recommendations

In response to the concerns discussed above, the following specific actions are recommended:

Intensify outreach to employers and employees

Industry and the research community are in great need of guidance concerning worker exposures to CNTs. Some studies have found elevated exposures in facilities that manufacture CNTs (Baron et al. 2003; Bello et al. 2008; Dahm et al. 2011; Han et al. 2008; Lee et al. 2010; Tsai et al. 2009b) and those that incorporate CNTs into devices (Bello et al. 2009; Cena and Peters 2011; Dahm et al. 2011). Unfortunately, these studies did not evaluate exposures in plants or laboratories subject to TSCA or REACH, so it is difficult at this time to assess either legislations impact on protecting worker health. It is likely that both approaches are having a positive effect on reducing worker exposures in the absence of specific exposure limits, but it is also likely that exposures have not been reduced to the lowest possible level, as would be required for any CNTs that are in fact carcinogens.

It has been our experience that many employers likely have not yet measured their workers' exposures, and those that have made measurements likely are unsure whether their exposures should be of concern. It should be of concern, however, if workers are being exposed to what likely is a potent carcinogen. It is absolutely imperative that the occupational hygiene community

do all it can to ensure that exposure to CNTs is being effectively controlled in workplaces, so that life threatening diseases won't develop in humans.

Provide more nuanced CNT OELs to industry

The mass-based OELs are meant to be protective against the non-carcinogenic adverse health effects from exposure. Referring to CNTs, we should consider 1) what OEL is appropriate to be protective against cancer, remembering that 2) the number concentration corresponding to a mass-based OEL is highly variable depending on the fiber size (Schulte et al. 2012).

Based on the published studies reviewed above, a mass-based OEL *may* be appropriate for SWCNTs and short MWCNTs, based on the lack of current evidence for their carcinogenicity. This approach may prove to be short-sighted, however, if future toxicology and/or epidemiology studies prove otherwise, and is somewhat similar to the seemingly endless debates over the relative carcinogenicity of different asbestos fiber types, lengths and diameters. Although for a period of time the ACGIH had different TLVs for different asbestos fiber types, the occupational hygiene community has in effect made a collective decision to avoid these arguments with respect to asbestos and to issue a single OEL for all asbestos types (but not all fiber lengths). Whether or not this is the proper approach for CNTs must be carefully considered.

In any case, the precautionary approach of reducing exposures to the lowest practical level may need to be applied to long MWCNTs ($d > 15$ nm, $L > 2$ μm), and, thus they may specifically be exempt from any mass-based CNT OEL. Given the uncertainties in health risk, exposure to all CNTs should be controlled to the lowest possible level. Any discussion of a CNT OEL should include statements that long MWCNTs should not be used unless absolutely necessary, according to current toxicological evidence discussed above, and then if and only if engineering and administrative controls are available to reduce exposure to the lowest possible level.

Although workers manufacturing such CNTs and incorporating them into devices are at the most risk, this precautionary approach shall be applied to all phases of a product's life.

Broaden the discussion

It is critical that decisions regarding the setting of OELs for CNTs involve all parties that have a role in this process. At a minimum in the United States, this should include NIOSH, OSHA, The American Conference of Governmental Hygienists (ACGIH), the American Industrial Hygiene Association (AIHA), and representatives from industry and labor. Appropriate representatives from other countries and areas involved in CNT research and manufacturing (European Union, China, Taiwan, Japan, Korea, *etc*.) should be included for a global perspective. The goals of these discussions should be 1) the setting of a consensus OEL (or OELs) for CNTs, and 2) agreement on a measurement method to be used for evaluating exposures for comparison to the OEL. Meeting these goals likely will require further research, discussed below.

Encourage further research

CNTs often are found as large bundles. However, the stability of these bundles is not well understood. Methods to study the stability of such bundles need to be developed. This method development would be done ideally in parallel with studies on the toxicity of such bundles so that a decision can be made whether bundles should be treated as the sum of many individual fibers or if only the number of free fibers need to be taken into account by a fiber count OEL. Until such a method is developed and tested, the proper procedure will remain a challenge. A conservative approach is to assume that individual fibers can be released into the surrounding tissue after such a bundle was deposited in the lungs; this would lead to the counting of individual fibers inside bundles.

Research is needed into the development of a reliable, cost-effective method to measure exposure to CNTs. Such a method likely would involve the direct collection of an air sample onto a filter or TEM grid, followed by a standardized fiber counting procedure, or a direct reading device that can measure fiber mass and/or volume. Research is needed in both of these areas. Several different techniques are available to directly deposit particles on TEM grids (diffusion, electrostatic and thermal deposition) and the method that best deposits particles of all relevant sizes should be determined. With regard to counting, it would be highly desirable to develop an automated method to scan grids and identify, count and size fibers, since manual counting and sizing is a very costly procedure.

Additional recommendations

CNT Safety Data Sheets, which to date have been seriously deficient (Eastlake et al. 2012), should include sufficient information to communicate the potential hazards discussed in this article. Efforts to prevent release to the environment should also be implemented. Information should also be provided to handlers of wastes containing these materials, including but not limited to personal protective equipment (respirator cartridges, disposable lab coats, gloves, etc.), cleaning wipes, and used air filters from exhaust systems. Manufacturers incorporating any CNT into products should consider appropriate warnings to users, and all products incorporating possibly a more toxic type, e.g. long MWCNTs, should include appropriate warning labels.

CNTs, of course, are only one category among many other nanomaterials either in current use or undergoing research for future use. It is likely that OELs will be needed for many of these materials; some of the difficult issues discussed here are unique to CNTS due to their being fibers, but the use of mass metrics will always present difficulties for nanomaterials. Precisely because they are so small, nanoparticle mass concentrations are typically very low, and masked

by the presence of other, larger particles in the same sample. Thus, we can expect significant difficulties in setting all OELs for nanomaterials. Nonetheless they will be needed, and the occupational health community needs to face this challenge head on.

Conclusion

The association between asbestos exposure and mesothelioma was established more than fifty years ago, but the mesothelioma epidemic continues. An estimated 107,000 people worldwide die from this disease every year; many of those now dying from mesothelioma are family members of the worker who had the primary exposure (Markowitz 2015). It is imperative that this disaster not be seen with other high aspect ratio particles such as CNTs. Strong action needs to be taken to minimize exposures to CNTs type 7 specifically and CNTs in general, and any CNT OEL should be consistent with the need to minimize exposures. The conclusions of Schulte, *et al*., (Schulte et al. 2012) are worth repeating:

> In the evolution of human civilizations, learning from the history and not repeating it has been a key guiding principle. Society can learn from how asbestos was inappropriately considered and not make the same mistake with CNTs. It is possible to safely realize the benefits of CNTs, but it will require rigorous and timely actions. The time to act is now.

Funding

No funding was received in connection with this manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

523

ACGIH (2012) 2012 TLVs and BEIs - based on the documentation of the threshold limit values for chemical substances and physical agents & biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Ali-Boucetta H, Nunes A, Sainz R, Herrero MA, Tian B, Prato M, Bianco A, Kostarelos K (2013) Asbestos-like pathogenicity of long carbon nanotubes alleviated by chemical functionalization. Angewandte Chemie (International Ed.In English) 52(8):2274-2278.

Baron PA, Maynard AD, Foley M (2003) Evaluation of aerosol release during the handling of unrefined single walled carbon nanotube material. Cincinnati: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. NIOSH DART-02-191 Rev.1.1.

Bello D, Hart AJ, Ahn K, Hallock M, Yamamoto N, Garcia EJ, Ellenbecker MJ, Wardle BL (2008) Particle exposure levels during CVD growth and subsequent handling of vertically-aligned carbon nanotube films. Carbon 46:974-981.

Bello D, Wardle BL, Yamamoto M, Guzman de Villoria R, Garcia EJ, Hart AJ, Ahn K, Ellenbecker MJ, Hallock M (2009) Exposure to nanoscale particles and fibers during machining of hybrid advanced composites containing carbon nanotubes. J Nanopart Res 11(1):231-249.

Birch EM, Wang C, Fernback JE, Feeng HA, Birch QT, Dozier AK (2016) Draft Report, Analysis of Carbon Nanotubes and Nanofibers on Mixed Cellulose Ester Filters by Transmission Electron Microscopy. Cincinnati: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.

Boxall AB, Tiede K, Chaudhry Q (2007) Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health? Nanomed (Lond) 2(6):919-927.

BSI (2007) Nanotechnologies - Part 2: Guide to safe handling and disposal of manufactured nanomaterials. British Standards Institute. Report No. 978 0 580 60832 2.

Cena L, Peters T (2011) Characterization and Control of Airborne Particles Emitted During Production of Epoxy/Carbon Nanotube Nanocomposites. J Occup Environ Hyg 8:86-92.

Commission of the European C. 2007. REACH. Commission of the European Communities.

Dahm M, Evans D, Schubauer-Berigan M, Birch M, Fernback J (2011) Occupational Exposure Assessment in Carbon Nanotube and Nanofiber Primary and Secondary Manufacturers. Ann Occup Hyg 56(5):542-556.

DOE (2008) Nanoscale Science Research Center: approach to nanomaterial ES&H, Rev.3a. Department of Energy.

Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, Alexander A (2006) Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. Toxicol Sci 92(1):5-22.

Eastlake A, Hodson L, Geraci C, Crawford C (2012) A critical evaluation of material safety data sheets (MSDSs) for engineered nanomaterials. Chem Health Saf 19(5):1-8.

EC (2008) Code of conduct for responsible nanosciences and nanotechnologies research. European Commission.

Ellenbecker MJ, Tsai SJ (2015) Health and Safety Considerations for Working with Engineered Nanoparticles. Wiley Interscience.

Ema M, Gamo M, Honda K (2016) A review of toxicity studies of single-walled carbon nanotubes in laboratory animals. Reg Toxic Pharmac 74:22.

EPA (2017) Significant New Use Rules on Certain Chemical Substances. Washington D.C.: Environmental Protetion Agency.

570 EU (2004) On the protection of workers from the risks related to exposure to carcinogens or mutagens
571 at work. European Union. Directive 2004/37/EC.
572 Gao Z, Varela JA, Groc L, Lounis B, Cognet L (2016) Toward the suppression of cellular toxicity from
573 single-walled carbon nanotubes. Biomater Sci 4(2):230-244.
574 Golanski L, Guiot A, Tardif F (2010) Experimental evaluation of individual protection devices against
575 different types of nanoaerosols: graphite, TiO2, and Pt. J Nanopart Res 12:83-89.
576 Grosse Y, Loomis D, Guyton KZ, Laugy-Secretan B, El Ghissassi F, Bouvard V, Bernbrahim-Tallea L, Guha
577 N, Scoccianti C, Mattock H, Straiff K (2014) Carcinogenicity of fluoro-edenite, silicon carbide
578 fibres and whiskers, and carbon nanotubes. Lancet Oncol 15(13):1427-1428.
579 Han JH, Lee EJ, Lee JH, So KP, Lee YH, Bae GN, Lee SB, Ji JH, Cho MH, Yu J (2008) Monitoring multiwalled
580 carbon nanotube exposure in carbon nanotube research facility. Inhal Toxicol 20(8):741-749.
581 IARC (2017) Some Nanomaterials and Some Fibres. Lyon: International Agency for Research on Cancer.
582 Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 111.
583 Kisin ER, Murray AR, Keane MJ, Shi XC, Schwegler-Berry D, Gorelik O, Arepalli S, Castranova V, Wallace
584 WE, Kagan VE, Shvedova AA (2007) Single-walled carbon nanotubes: geno- and cytotoxic effects
585 in lung fibroblast V79 cells. J Toxicol Environ Health A 70(24):2071-2079.
586 Kisin ER, Murray AR, Sargent L, Lowry D, Chirila M, Siegrist KJ, Schwegler-Berry D, Leonard S, Castranova
587 V, Fadeel B, Kagan VE, Shvedova AA (2011) Genotoxicity of carbon nanofibers: Are they
588 potentially more or less dangerous than carbon nanotubes or asbestos? Toxicol Appl Pharmacol
589 252(1):1-10.
590 Kuempel ED, Jaurand M, Møller P, Morimoto Y, Kobayashi N, Pinkerton KE, Sargent LM, Vermeulen RCH,
591 Fubini B, Kane AB (2016) Evaluating the mechanistic evidence and key data gaps in assessing the
592 potential carcinogenicity of carbon nanotubes and nanofibers in humans. Crit Rev Toxicol
593 58(1):1-58.
594 Lam CW, James JT, McCluskey R, Arepalli S, Hunter RL (2006) A review of carbon nanotube toxicity and
595 assessment of potential occupational and environmental health risks. Crit Rev Toxicol 36(3):189-
596 217.
597 Lee JH, Lee SB, Bae GN, Jeon KS, Yoon JU, Ji JH, Sung JH, Lee BG, Yang JS, Kim HY, Kang CS, Yu IJ (2010)
598 Exposure assessment of carbon nanotube manufacturing workplaces. Inhal Toxicol 22(5):369-
599 381.
600 Legramante JM, Sacco S, Crobeddu P, Magrini A, Valentini F, Palleschi G, Pallante M, Balocchi R, Iavocoli
601 I, Bergamaschi A, Galante A, Campagnolo L, Pietrolusti A (2012) Changes in cardiac autonomic
602 regulation after acute lung exposure to carbon nanotubes: implications for occupational
603 exposure. J Nanomat 212:Article ID 397206.
604 Li Z, Hulderman T, Salmen R, Chapman R, Leonard SS, Young SH, Shvedova AA, Luster MI, Simeonova PP
605 (2007) Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environ
606 Health Perspect 115(3):377-382.
607 Ma-Hock L, Burkhardt S, Strauss V, Gamer AO, Wiench K, van Ravenzwaay B, Landsiedel R (2009)
608 Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model
609 substance. Inhal Toxicol 21(2):102-118.
610 Markowitz S (2015) Asbestos-related lung cancer and malignant mesothelioma of the pleura: Selected
611 current issues. Semin Respir Crit Care Med 36:13.
612 Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Schwegler-Berry D, Castranova V, Porter DW
613 (2010) Distribution and persistence of pleural penetrations by multi-walled carbon nanotubes.
614 Part Fibre Toxicol 7:28.
615 Muller J, Delos M, Panin N, Rabolli V, Huaux F, Lison D (2009) Absence of carcinogenic response to
616 multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. Toxicol Sci
617 110(2):442-448.

Muller J, Huaux F, Moreau N, Misson P, Heilier JF, Delos M, Arras M, Fonseca A, Nagy JB, Lison D (2005) Respiratory toxicity of multi-wall carbon nanotubes. Toxicol Appl Pharm 207(3):221-231.

Nagai H, Okazaki Y, Chew SH, Misawa N, Yamashita Y, Akatsuka S, Ishiraha T, Yamashita K, Yoshikawa Y, Yasui H, Jiang L, Ohara H, Takahashi T, Ichihara G, Kostarelos K, Miyata Y, Shinohara H, Toyokunia S (2011) Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. Proc Natl Acad Sci U S A 108(49):E1330-E1338.

Nakanishi J. 2011. Risk assessment of manufactured nanomaterials: "approaches" - overview of approaches and results; carbon nanotubes (CNTs). New Energy and Industrial Technology Development Organization (NEDO).

NCI (2008) Working with nanomaterials. National Cancer Institute at Frederick.

NIOSH (1994) Asbestos by TEM. Cincinnati: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. Method 7402, Issue 2.

NIOSH (2003) Diesel particulate matter (as elemental carbon). Cincinnati: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. Method 5040, Issue 3.

NIOSH (2012) General safe practices for working with engineered nanomaterials in research laboratories. Cincinnati: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. Pub. No. 2012-147.

NIOSH (2013) Current intelligence bulletin 65 - occupational exposure to carbon nanotubes and nanofibers. Cincinnati: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. Pub. No. 2013-145.

Oberdörster G, Castranova V, Asgaharian B, Sayre P (2015) Inhalation exposure to carbon nanotubes (CNT) and carbon nanofibers (CNF): Methodology and dosimetry. J Toxicol Env Health B 18(3-4):121-212.

OECD (2016a) Multiwalled Carbon Nanotubes (MWCNTs): Summary of the Dossier. Paris: Organization for Economic Cooperation and Development. OECD Environment, Health and Safety Publications, Series on the Safety of Manufactured Nanomaterials. No. 68.

OECD (2016b) Single Walled Carbon Nanotubes (SWCNTs): Summary of the Dossier. Paris: Organization for Economic Cooperation and Development. OECD Environment, Health and Safety Publications, Series on the Safety of Manufactured Nanomaterials. No. 70.

Ong LC, Chung FFL, Tan YF, Leong CO (2016) Toxicity of single-walled carbon nanotubes. Arch Toxicol 90:103-118.

OSHA (1994) Occupational exposure to asbestos. Washington, DC: Occupational Safety and Health Administration. 29 CFR 1910.1001, 1915.1001, 1926.58.

Pacurari M, Lowe K, Tchounwou PB, Kafoury R (2016) A review on the respiratory system toxicity of carbon nanoparticles. Int J Environ Res Pub Health 13(1).

Pauluhn J (2010) Multi-walled carbon nanotubes (Baytubes): Approach for derivation of occupational exposure limit. Reg Toxic Pharmac 57(1):78-89.

Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WAH, Seaton A, Stone V, Brown S, MacNee W, Donaldson K (2008) Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nature Nanotechnol 3(7):423-428.

Rittinghausen S, Hackbarth A, Creutzenberg O, Ernst H, Heinrich U, Leonhardt A, Schaudien D (2014) The carcinogenic effect of various multi-walled carbon nanotubes (MWCNTs) after intraperitoneal injection in rats. Part Fibre Toxicol 11:59.

Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, Moss OR, Wong BA, Dodd DE, Andersen ME, Bonner JC (2009) Inhaled carbon nanotubes reach the subpleural tissue in mice. Nat Nanotechnol 4(11):747-751.

666 Schinwald A, Murphy FA, Prina-Mello A, Poland CA, Byrne F, Movia D, Glass JR, Dickerson JC, Schultz DA,
667 Jeffree CE, MacNee W, Donaldson K (2012) The threshold length for fiber-induced acute pleural
668 inflammation: shedding light on the early events in asbestos-induced mesothelioma. Toxicol Sci
669 128(2):461-470.
670 Schulte PA, Kuempel ED, Zumwalde RD, Geraci CL, Schubauer-Berigan MK, Castranova V, Laura Hodson
671 L, Murashov V, Matthew M. Dahm MM, Ellenbecker MJ (2012) Focused action to protect carbon
672 nanotube workers. Am J Ind Med 55(5).
673 Service RF (1998) Nanotubes: The next asbestos? Science 281(5379):942.
674 Shvedova AA, Castranova V, Kisin ER, Schwegler-Berry D, Murray AR, Gandelsman VZ, Maynard A, Baron
675 P (2003) Exposure to carbon nanotube material: Assessment of nanotube cytotoxicity using
676 human keratinocyte cells. J Toxicol Environ Health A 66(20):1909-1926.
677 Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S,
678 Schwegler-Berry D and others (2005) Unusual inflammatory and fibrogenic pulmonary
679 responses to single-walled carbon nanotubes in mice. Am J Physiol Lung Cell Mol Physiol
680 289(5):698-708.
681 Shvedova AA, Fabisiak JP, Kisin ER, Murray AR, Roberts JR, Tyurina YY, Antonini JM, Feng WH,
682 Kommineni C, Reynolds J, Barchowski A, Castranova V, Kagan VE (2008a) Sequential exposure to
683 carbon nanotubes and bacteria enhances pulmonary inflammation and infectivity. Am J Respir
684 Cell Mol Biol 38(5):579-590.
685 Shvedova AA, Kisin ER, Murray AR, Johnson VJ, Gorelik O, Arepalli S, Hubbs AF, Mercer R, Keohavong P,
686 Sussman N and others (2008b) Inhalation vs. aspiration of single-walled carbon nanotubes in
687 C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. Am J Physiol Lung Cell
688 Mol Physiol 295(4):L552-L565.
689 Shvedova AA, Kisin ER, Porter D, Schulte P, Kagan VE, Fadeel B, Castranova V (2008c) Mechanisms of
690 pulmonary toxicity and medical applications of carbon nanotubes: Two faces of Janus?
691 Pharmacol Ther 121(2):192-204.
692 Siegrist KJ, Reynolds SH, Kashon ML, Lowry DT, Dong C, Hubbs AF, Young SH, Salisbury JL, Porter DW,
693 Benkovic SA and others (2014) Genotoxicity of multi-walled carbon nanotubes at occupationally
694 relevant doses. Part Fibre Toxicol 11(6).
695 Simeonova PP (2009) Update on carbon nanotube toxicity. Nanomed 4(4):373-375.
696 Song ZM, Wang L, Chen N, Cao A, Liu Y, Wang H (2016) Biological effects of agglomerated multi-walled
697 carbon nanotubes. Coll Surf B Biointerfaces 142:65-73.
698 SUVA. 2011. Grenzwerte am arbietsplatz 2011 (Occupational exposure limits 2011). Lucerne: Swiss
699 Accident Insurance Funds (SUVA).
700 Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J (2008) Induction of
701 mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. J
702 Toxicol Sci 33(1):105-116.
703 Tsai SJ, Ashter A, Ada E, Mead J, Barry C, Ellenbecker MJ (2008a) Airborne nanoparticle release
704 associated with the compounding of nanocomposites using nanoalumina as fillers. Aerosol Air
705 Qual Res 8(2):160-177.
706 Tsai SJ, Ashter A, Ada E, Mead J, Barry C, Ellenbecker MJ (2008b) Control of airborne nanoparticle
707 release during compounding of polymer nanocomposites. Nano 3(4):1-9.
708 Tsai SJ, Ada E, Isaacs J, Ellenbecker MJ (2009a) Airborne nanoparticle exposures associated with the
709 manual handling of nanoalumina and nanosilver in fume hoods. J Nanopart Res 11(1):147-161.
710 Tsai SJ, Hofmann M, Hallock M, Ada E, Kong J, Ellenbecker MJ (2009b) Characterization and evaluation of
711 nanoparticle release during the synthesis of single-walled and multi-walled carbon nanotubes by
712 chemical vapor deposition. Environ Sci Technol 43(15):6017-6023.

713 Tsai SJ, White D, Rodriguez H, Munoz C, Huang CY, Tsai CJ, Barry C, Ellenbecker M (2012) Exposure
714 assessment and engineering control strategies for airborne nanoparticles: an application to
715 emissions from nanocomposite compounding processes. J Nanopart Res 14(7):989.
716 van Broekhuizen P, Dorbeck-Jung B (2013) Exposure limit values for nanomaterials - capacity and
717 willingness of users to apply a precautionary approach. J Occup Environ Hyg 10(1):46-53.
718 van Broekhuizen P, van Broekhuizen F, Cornelissen R, Reijnders L (2012) Workplace exposure to
719 nanoparticles and the application of provisional nanoreference values in times of uncertain risk.
720 J Nanopart Res 14(4):770.
721 Vietti G, Lison D, van den Brule S (2016) Mechanisms of lung fibrosis induced by carbon nanotubes:
722 towards an Adverse Outcome Pathway (AOP). Part Fibre Toxicol 13(1):11.
723 Wang X, Xia T, Duch MC, Ji X, Zhang H, Li R, Sun B, Lin S, Meng H, Liao YP, Wang M, Song TB, Yang Y,
724 Hersam MC, Nel AE (2012) Pluronic F108 coating decreases the lung fibrosis potential of
725 multiwall carbon nanotubes by reducing lysosomal injury. Nano Lett 12:3050-3061.
726 Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GAM, Webb TR (2004) Comparative pulmonary
727 toxicity assessment of single-wall carbon nanotubes in rats. Toxicol Sci 77(1):117-125.
728 Wick O, Manser P, Limbach LK, Dettlaf-Weglikowska U, Krumeich F, Roth S, Stark A, Bruinink A (2007)
729 The degree and kind of agglomeration affect carbon nanotube cytotoxicity. Toxicol Lett
730 168(2):121-131.
731 Wood JP (2000) Containment in the pharmaceutical industry. London, UK: CRC Press.

732