



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](https://doi.org/10.1039/c8en00518d)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Grieger, K. D., Bossa, N., Levis, J. W., von Borries, K. J. F., Strader, P., Cuchiara, M., Hendren, C. O., Hansen, S. F., & Jones, J. L. (2018). Application and testing of risk screening tools for nanomaterial risk analysis. *Environmental Science: Nano*, 5(8), 1844-1858. <https://doi.org/10.1039/c8en00518d>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 The Difficulties in Establishing an Occupational Exposure Limit for Carbon Nanotubes

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

3 M. Ellenbecker
4 Toxics Use Reduction Institute
5 University of Massachusetts Lowell
6 Lowell, MA 01854, USA

7
8 S-J. Tsai
9 Department of Environmental and Radiological Health Science
10 Colorado State University
11 Fort Collins, CO 80523-1681, USA

12
13 M. Jacobs
14 Lowell Center for Sustainable Production
15 University of Massachusetts Lowell
16 Lowell, MA 01854, USA

17
18 M. Riediker
19 Institute for Work and Health
20 Lausanne CH-1066, Switzerland

21
22 T. Peters
23 Department of Occupational and Environmental Health
24 The University of Iowa
25 Iowa City, IA 52242, USA

26
27 S. Liou
28 National Institute of Environmental Health Sciences
29 Zhunan Town, 35053, Taiwan

30
31
32 A. Avila:
33 Dept. of Electrical and Electronic Engineering and Centro de Microelectrónica (CMUA),
34 Universidad de los Andes, Bogotá 11001, Colombia

35
36 S. Foss Hansen
37 Department of Environmental Engineering
38 Technical University of Denmark
39 Lyngby, Denmark

40
41
42 Corresponding author:

43 M. Ellenbecker
44 ellenbec@turi.org
45 Phone: 1.978.934.3272
46 Fax: 1.978.934.3050
47

48 **Abstract**

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

65 **Introduction**

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

68 2007; Donaldson et al. 2006; Kisin et al. 2007; Kisin et al. 2011; Lam et al. 2006; Legramante et
69 al. 2012; Li et al. 2007; Ma-Hock et al. 2009; Muller et al. 2005; Shvedova et al. 2003; Shvedova
70 et al. 2008a; Shvedova et al. 2005; Shvedova et al. 2008b; Shvedova et al. 2008c; Simeonova
71 2009; Warheit et al. 2004) outlines the growing concerns regarding the toxicity of CNTs. Recent
72 review papers (Ema et al. 2016; Gao et al. 2016; Kuempel et al. 2016; Ong et al. 2016; Pacurari
73 et al. 2016; Siegrist et al. 2014; Vietti et al. 2016) provide an excellent overview of the current
74 knowledge regarding adverse health effects of single-walled carbon nanotubes (SWCNTs) and
75 multi-walled carbon nanotubes (MWCNTs). The Organization for Economic Cooperation and
76 Development (OECD) has recently published comprehensive summaries of the environmental
77 health and safety aspects of both MWCNTs (OECD 2016a) and SWCNTs (OECD 2016b).
78 Rodent studies have found an acute inflammatory response, granulomas, fibrosis, and decreased
79 rates of respiration and bacterial clearance from the lungs. Importantly, the National Institute for
80 Occupational Safety and Health (NIOSH) (NIOSH 2013) concluded that "...in animal studies
81 where CNTs were compared with other known fibrogenic materials (e.g., silica, asbestos,
82 ultrafine carbon black), the CNTs were of similar or greater potency, and the effects, including
83 fibrosis, developed soon after exposure and persisted." Adverse impacts on other organ systems,
84 including cardiac inflammation, have also been found (NIOSH 2013). Such a wide range of
85 acute and chronic health effects associated with CNTs, particularly the strong fibrogenic
86 potential, are reason enough for concern – but even more serious concerns have arisen. Almost
87 twenty years ago, the morphological similarity between CNTs and other fibrous materials, such
88 as asbestos, raised concerns as to whether exposure to CNTs could cause lung cancer and/or
89 mesothelioma (Service 1998). Research followed, and two groups observed asbestos-like effects
90 in short-term bioassays when MWCNTs were injected intraperitoneally into mice (Poland et al.

91 2008; Takagi et al. 2008); subsequently, Ryman-Rasmussen *et al.* (Ryman-Rasmussen et al.
92 2009) found that inhaled MWCNTs reached the subpleura of mice and Mercer *et al.* (Mercer et
93 al. 2010) found that they penetrated the intrapleural space. Additional research has for the most
94 part confirmed the results of the first studies (Muller et al. 2009; Nagai et al. 2011; Rittinghausen
95 et al. 2014; Schinwald et al. 2012), while others were negative (Muller et al. 2009).

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

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

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

129 This commentary focuses on the issue of setting appropriate occupational exposure levels
130 (OELs) for CNTs, although many issues must be addressed, including exposure assessment
131 methodologies and effective exposure control strategies. We first describe the OELs suggested
132 by government agencies and companies; at this time there are no regulatory OELs specific to
133 CNTs. We then discuss important issues that must be addressed in the setting of an OEL for
134 CNTs, including the more fundamental question about the appropriateness of OELs for
135 suspected carcinogens. We close with some recommendations for actions we believe should be
136 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/cm³ (f/cm³), 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/m³, 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/m³ for SWCNTs and 80 µg/m³ 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 $1 \mu\text{g/m}^3$ 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

181 liters per minute (lpm).” Regarding health effects, for a 45-year lifetime exposure at the REL,
182 NIOSH developed “maximum likelihood estimates” of 2.4 – 33% for “minimal lung effects” and
183 0.23 – 10% for “slight or mild lung effects” as. The CIB concluded that “NIOSH does not
184 consider a 10% estimated excess risk over a working lifetime to be acceptable for these early-
185 stage lung effects, and the REL is set at the optimal limit of quantification (LOQ) of the
186 analytical method carbon (NIOSH method 5040).”

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

192 To summarize, various entities have recommended both mass-based and number-based OELs
193 for CNTs, as shown in Table 1. The number-based recommendations all are consistent with the
194 strictest asbestos OEL of 0.01 f/cm³, whereas the mass-based recommendations range from 1 –
195 80 µg/m³.

196

197 **Advantages and Disadvantages of a Mass-Based OEL for CNTs**

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

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

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

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

226 dimensions to a mass concentration of $7 \mu\text{g}/\text{m}^3$ (this was the original proposed REL of NIOSH).
227 Adjusting their conversions to $1 \mu\text{g}/\text{m}^3$, this corresponds to $0.01 \text{ fibers}/\text{cm}^3$ for a very large fiber
228 (2,110 nm diameter x 10,000 nm length) and $300,000 \text{ fibers}/\text{cm}^3$ for a very small fiber (2 nm x
229 500 nm). These fiber concentrations range from lower than the asbestos PEL of $0.1 \text{ f}/\text{cm}^3$ to
230 much higher than the PEL (OSHA 1994).

231

232 **Advantages and Disadvantages of a Number-Based OEL for CNTs**

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

245

246 **Possible Variations between Different CNT Types**

247 A one-size-fits-all OEL is unlikely to adequately protect workers because the literature
248 suggests that there may be important differences in toxicological response among types of CNTs.
249 In various studies, single wall carbon nanotubes (SWCNTs) have not been found to cause
250 mesothelioma (Kuempel et al. 2016). In addition, thin ($d < 15$ nm) and short [$L < 1$ μ m
251 according to Muller et al. (Muller et al. 2009) and $L < 5$ μ m according to Schinwald et al.
252 (Schinwald et al. 2012) MWCNTs do not cause mesothelioma. SWCNTs and thin MWCNTs,
253 when examined microscopically, tend to curl and form bundles which are not fiber-shaped.
254 Presumably, this shape enhances their clearance from the pulmonary region by phagocytosis.
255 Likewise, very short MWCNTs may be cleared effectively by macrophages (Rittinghausen et al.
256 2014). As an added complication, Nagai et al. (Nagai et al. 2011) found that very thick
257 MWCNTs ($d > 150$ nm) were less carcinogenic than thinner ones; however, there is evidence
258 that such large-diameter tubes are not important commercially.

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

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

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

278

279 **Agglomerates vs. Individual Fibers**

280 Another significant complication that is not addressed by using a mass-based OEL is that
281 airborne CNTs may exist as individual fibers or as agglomerates or bundles of fibers. The state
282 of agglomeration can influence both the respiratory deposition pattern of the inhaled fibers and
283 the toxicological response. Researchers have found varied changes in toxicity when CNTs
284 agglomerate. For example, Gao et al. (Gao et al. 2016) found that “it clearly appears that
285 aggregation of SWCNTs should be avoided and that nanotube individualization is a key
286 parameter to minimize cellular toxicity.” Wick et al. (Wick et al. 2007) found that agglomerated
287 MWCNTs were more toxic than well-dispersed ones. On the other hand, end effects that depend
288 on the fibrotic nature of CNTs (fibrosis and mesothelioma) should presumably be ameliorated by
289 the formation of non-fiber-shaped agglomerates (Kuempel et al. 2016). Song et al. (Song et al.
290 2016) summarized the current muddled state of the research on this topic thus: “more efforts
291 should be paid to study the biological effects of agglomeration.” In any case, a significant

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

294 From an occupational hygiene viewpoint, agglomeration complicates the use of number-
295 based OELs. Counting schemes need to be developed to address this issue; it is likely that an
296 approach similar to that used for high aspect ratio particles such as bundles of asbestos fibers,
297 where clearly-identified fiber “ends” in fiber bundles are counted, may be needed. NIOSH in
298 2016 published a draft analytical method titled “Analysis of Carbon Nanotubes and Nanofibers
299 on Mixed Cellulose Ester Filters by Transmission Electron Microscopy” (Birch et al. 2016),
300 which was a modification to NIOSH NMAM 7402, asbestos by TEM (NIOSH 1994). It is an
301 initial attempt to develop an approach in the United States.

302

303 **Actions by Other Government Agencies**

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

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

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

329

330 N&N research activities should be safe, ethical and contribute to sustainable
331 development serving the sustainability objectives of the Community as well as
332 contributing to the United Nations' Millennium Development Goals. They should
333 not harm or create a biological, physical or moral threat to people, animals, plants
334 or the environment, at present or in the future.

335 N&N research activities should be conducted in accordance with the
336 precautionary principle, anticipating potential environmental, health and safety
337 impacts of N&N outcomes and taking due precautions, proportional to the level of
338 protection, while encouraging progress for the benefit of society and the
339 environment.

340 Governance of N&N research activities should be guided by the principles of
341 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

389 represents a case of evident exposure to workers, since in every case there is evidence of toxicity
390 before the 1A designation and, in spite of that evidence, exposures were allowed sufficient to
391 lead to a statistically significant level of cancer. Recognizing this, the American Conference of
392 Governmental Industrial Hygienists states that for suspected human carcinogens, “worker
393 exposure by all routes should be carefully controlled to levels as low as possible...” (ACGIH
394 2012) Our goal should be that there is *never* a positive epidemiology study for MWCNTs (or any
395 other engineered nanoparticle, for that matter).

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

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

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

413

414 **Recommendations**

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

417 **Intensify outreach to employers and employees**

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

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

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

434 **Provide more nuanced CNT OELs to industry**

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

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

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

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

457 **Broaden the discussion**

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

468 **Encourage further research**

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

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

487 **Additional recommendations**

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

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

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

504

505 **Conclusion**

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

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

519 **Funding**

520 No funding was received in connection with this manuscript.

521 **Conflict of Interest**

522 The authors declare that they have no conflict of interest.

- 525 ACGIH (2012) 2012 TLVs and BEIs - based on the documentation of the threshold limit values for
526 chemical substances and physical agents & biological exposure indices. Cincinnati, OH: American
527 Conference of Governmental Industrial Hygienists.
- 528 Ali-Boucetta H, Nunes A, Sainz R, Herrero MA, Tian B, Prato M, Bianco A, Kostarelos K (2013) Asbestos-
529 like pathogenicity of long carbon nanotubes alleviated by chemical functionalization.
530 *Angewandte Chemie (International Ed. In English)* 52(8):2274-2278.
- 531 Baron PA, Maynard AD, Foley M (2003) Evaluation of aerosol release during the handling of unrefined
532 single walled carbon nanotube material. Cincinnati: Department of Health and Human Services,
533 Centers for Disease Control and Prevention, National Institute for Occupational Safety and
534 Health. NIOSH DART-02-191 Rev.1.1.
- 535 Bello D, Hart AJ, Ahn K, Hallock M, Yamamoto N, Garcia EJ, Ellenbecker MJ, Wardle BL (2008) Particle
536 exposure levels during CVD growth and subsequent handling of vertically-aligned carbon
537 nanotube films. *Carbon* 46:974-981.
- 538 Bello D, Wardle BL, Yamamoto M, Guzman de Villoria R, Garcia EJ, Hart AJ, Ahn K, Ellenbecker MJ,
539 Hallock M (2009) Exposure to nanoscale particles and fibers during machining of hybrid
540 advanced composites containing carbon nanotubes. *J Nanopart Res* 11(1):231-249.
- 541 Birch EM, Wang C, Fernback JE, Feeng HA, Birch QT, Dozier AK (2016) Draft Report, Analysis of Carbon
542 Nanotubes and Nanofibers on Mixed Cellulose Ester Filters by Transmission Electron
543 Microscopy. Cincinnati: Department of Health and Human Services, Centers for Disease Control
544 and Prevention, National Institute for Occupational Safety and Health.
- 545 Boxall AB, Tiede K, Chaudhry Q (2007) Engineered nanomaterials in soils and water: how do they behave
546 and could they pose a risk to human health? *Nanomed (Lond)* 2(6):919-927.
- 547 BSI (2007) Nanotechnologies - Part 2: Guide to safe handling and disposal of manufactured
548 nanomaterials. British Standards Institute. Report No. 978 0 580 60832 2.
- 549 Cena L, Peters T (2011) Characterization and Control of Airborne Particles Emitted During Production of
550 Epoxy/Carbon Nanotube Nanocomposites. *J Occup Environ Hyg* 8:86-92.
- 551 Commission of the European C. 2007. REACH. Commission of the European Communities.
- 552 Dahm M, Evans D, Schubauer-Berigan M, Birch M, Fernback J (2011) Occupational Exposure Assessment
553 in Carbon Nanotube and Nanofiber Primary and Secondary Manufacturers. *Ann Occup Hyg*
554 56(5):542-556.
- 555 DOE (2008) Nanoscale Science Research Center: approach to nanomaterial ES&H, Rev.3a. Department of
556 Energy.
- 557 Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, Alexander A (2006) Carbon nanotubes: a
558 review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicol Sci*
559 92(1):5-22.
- 560 Eastlake A, Hodson L, Geraci C, Crawford C (2012) A critical evaluation of material safety data sheets
561 (MSDSs) for engineered nanomaterials. *Chem Health Saf* 19(5):1-8.
- 562 EC (2008) Code of conduct for responsible nanosciences and nanotechnologies research. European
563 Commission.
- 564 Ellenbecker MJ, Tsai SJ (2015) Health and Safety Considerations for Working with Engineered
565 Nanoparticles. Wiley Interscience.
- 566 Ema M, Gamo M, Honda K (2016) A review of toxicity studies of single-walled carbon nanotubes in
567 laboratory animals. *Reg Toxic Pharmacol* 74:22.
- 568 EPA (2017) Significant New Use Rules on Certain Chemical Substances. Washington D.C.: Environmental
569 Protection 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, Cagnet 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, Scocciati 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.

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

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

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

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

628 NIOSH (1994) Asbestos by TEM. Cincinnati: Department of Health and Human Services, Centers for
629 Disease Control and Prevention, National Institute for Occupational Safety and Health. Method
630 7402, Issue 2.

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

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

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

640 Oberdörster G, Castranova V, Asgaharian B, Sayre P (2015) Inhalation exposure to carbon nanotubes
641 (CNT) and carbon nanofibers (CNF): Methodology and dosimetry. *J Toxicol Env Health B* 18(3-
642 4):121-212.

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

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

649 Ong LC, Chung FFL, Tan YF, Leong CO (2016) Toxicity of single-walled carbon nanotubes. *Arch Toxicol*
650 90:103-118.

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

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

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

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

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

663 Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, Moss OR, Wong
664 BA, Dodd DE, Andersen ME, Bonner JC (2009) Inhaled carbon nanotubes reach the subpleural
665 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 arbeitsplatz 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, Dettlaff-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