A summary of pertinent comments received from Peer Reviewers on the November 2010 draft Current Intelligence Bulletin (CIB): *Occupational Exposure to Carbon Nanotubes and Nanofibers* along with the NIOSH response and subsequent changes to the final document. The complete text of submitted comments can be found at: http://www.cdc.gov/niosh/docket/archive/docket161A.html

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 1	**Question:** **A. Are there additional data that would better characterize the exposure to workers due to the handling of CNTs and CNFs, thus allowing an improved understanding of the overall risks posed by these materials?** **A1.** The document states (page 19) that there are limited data on the number of workers potentially exposed to CNT and CNF, and that the extent of exposure in workplace settings has not been well characterized. Citations to the open literature are provided; however, there are no detailed data from NIOSH's own program on monitoring of materials like carbon nanofibers in the workplace. According to NIOSH **(http://www.cdc.gov/niosh/topics/nantech/field.htm)**, since 2006 the Nanotechnology Field Research Team has been working to expand its knowledge and understanding of the potential health and safety risks that workers may encounter during the research, production, and use of engineered nanomaterials by conducting site visits.	**A1.** Relatively few studies have included personal monitoring, and we know of just one that has addressed exposure to complex mixtures. NIOSH researchers have conducted studies at carbon nanotube (CNT) research laboratories, pilot plants, and manufacturing facilities [Methner et al. 2010a, b; Dahm et al. 2011]. Studies were conducted	**A1.** Section 2 *Potential for Exposure* was updated to include recently published studies. Additional analysis of exposure data was incorporated.

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Peer Reviewer 1 (cont.)	These site visits include monitoring of the workplace air for nanomaterials. Please provide those monitoring data, and/or an aggregate set of numbers that represent several CNT sites if confidentiality of private sector sites is involved. This would enable a more realistic comparison of the potential hazards to the actual exposures to respirable fractions of CNTs and CNFs in the workplace. The raw data would also potentially be useful in EPA risk assessments of CNT and CNF Premanufacture Notices. The use of respirable mass as a dose metric is appropriate at this time. However, the risk assessment and associated analyses that form the bases of the REL may be in need of some amendment. Please consider the following points:	to determine whether airborne exposures occur and to assess the capabilities of various measurement techniques. A comprehensive study has also been conducted at a carbon nanofibers (CNF) manufacturing facility [Birch et al. 2011b, Birch 2011a, Evans et al. 2010]. Filter, sorbent, impactor, bulk, and microscopy samples, combined with direct-reading instruments for CO and aerosol measurement (particle number, size distribution, mass, and active surface area), provided complementary information. Samples were analyzed for organic and elemental carbon (OC and EC), metals, and polycyclic aromatic hydrocarbons	

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Peer Reviewer 1 (cont.)		(PAHs), with EC as a measure of CNF. Scanning electron microscopy (SEM) and transmission electron microscopy with energy dispersive x-ray spectroscopy (TEM-EDS) also were applied. Air dispersion of CNF was significant. Respirable EC area concentrations were about 6 to 68 times higher than outdoors. Personal breathing zone samples were up to 170 times higher. Area sample concentrations were not predictive of personal exposure to CNFs. Iron-rich soot, PAHs, and carbon monoxide were production byproducts. In addition to the CNF surveys, NIOSH researchers applied TEM, NIOSH 5040, and	

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Peer Reviewer 1 (cont.)		direct reading instruments to multiple field surveys on CNT/CNF. A paper summarizing their finding was recently published in the Annals of Occupational Hygiene [Dahm et al. 2011]. As found in previous studies [Birch et al. 2011, Evans et al. 2010], direct reading instruments were useful as indicators of background and byproduct aerosols but not particularly useful for CNT/CNF monitoring.	
	Question: **B. Is the use of Co levels in lungs for CNT lung burden estimations, per Pauluhn (2010a) preferable to the current CIB approach?**		
	B1. The CIB estimates the retained lung burden in rats from the Pauluhn study using the MPPD 2.0	**B1.** Agree that the Co tracer based	**B1.**Section A.6.1.2 has been added to provide

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Peer Reviewer 1 (cont.)	model, based on particle MMAD and GSD, assuming among other things that the deposition and clearance of the CNTs is equivalent to spherical particles with the equivalent MMAD and GSD. Alternatively, Pauluhn (2010a) used the "matrix-bound Co" in the CNTs to estimate lung burdens, which may provide more realistic estimates of CNT lung burdens. Is this a more data-driven method to estimate lung burdens, as opposed to the method used in the CIB which may contain more assumptions? The Ellinger and Pauluhn manuscript in preparation cited in the Pauluhn publication should be examined to validate the stability of the remaining Co in CNTs and other calculations used to arrive at lung burden estimates in this way.	measurements of CNT lung burden [Ellinger-Ziegelbauer and Pauluhn 2009; Pauluhn 2010a] provide useful information to which the model-based estimates can be compared.	these comparisons. The Co-tracer based estimates of CNT lung burden at the end of the 13-week inhalation exposure [Pauluhn [2010a] generally laid between the MPPD 2.0 deposited and retained lung dose estimates (Table A-10). This is consistent with the reduced lung clearance rate for CNT reported by Pauluhn [2010a]. The Co-tracer based estimate of CNT 90-d after the one 6-hr inhalation exposure at 11 mg/m^3 [Ellinger-Ziegelbauer and Pauluhn 2009] is also between the deposited and retained lung doses estimated by MPPD 2.0. However, the Co-tracer based estimate of the lung burden at 241 mg/m^3 is lower (by almost half) than the MPPD 2.0 retained lung burden estimate, which

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Peer Reviewer 1 (cont.)			implies a greater clearance of the deposited CNT than poorly soluble spherical particles, is inconsistent with Pauluhn [2010a], and suggests some error in that measurement.
	Question: **C. Is there a need to cite cardiovascular effects literature related to these nanomaterials in greater detail?**		
	C1. The research needs on page 60 of the CIB include a focus on additional research on cardiovascular effects of CNTs. However, the current literature on CNTs does not appear to be fully incorporated into the CIB. Please consider publications such as the following and provide an analysis of what is known now about cardiovascular effects of CNTs: Erdely, et al (2009); Li, et al (2007), Legramante, et al (2009), and Nurkiewicz et al (2008).	**C1.** A discussion of systemic responses (including cardiovascular) from pulmonary exposure to SWCNT and MWCNT was added.	**C1.** The section on *Research Needs* was clarified. Section 3.4 was added to the document to summarize systemic effects from SWCNT and MWCNT.

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Peer Reviewer 1 (cont.)	**Question:** **D. Are there additional information on CNT and CNF that NIOSH should consider for the NIOSH CIB?** **D1.** The only support cited for inclusion of CNFs in the document is an abstract from a yet-to-be published journal article on CNFs. It would be helpful to have the peer-reviewed manuscript available to support the abstract.	**D1.** Agree	**D1.** The journal article by Murray et al. [2012] *Factoring in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos,* Particle and Fibre Toxicology is now cited in the CIB that documents lung inflammation and fibrosis observed in animals exposed to CNF. The animal inhalation study with CNF reported by DeLorme et al. 2012 is also cited.

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Peer Reviewer 1 (cont.)	Question: **E. Is the benchmark dose modeling approach appropriate for the derivation of an OEL, as opposed to using NOAELs from the two subchronic inhalation studies?** **E1.** As pointed out in section A.2.1 (Rodent dose-response data), "In general, the CNT animal studies have limited data, with few (4-20) animals per dose group and sparse dose group spacing...Some of these studies just meet the minimum data criteria for BMD estimation, i.e., a graded monotonic response with dose and at least two dose groups in addition to the unexposed (control) group", for which criteria they credit EPA's Benchmark Dose Technical Guidance Document of 2000 (EPA/630/R-00/001). This was an External Review Draft, which has not yet made it out of Final Review (although EPA/630/R-00-0001F May 2008 exists). **E2.** On page 107 the text mentions a feature of the dichotomous data that severely limits considering any modeling, let alone deriving a BMD(L)x, namely, that only one dose displayed a partial response. This feature was displayed by both the subchronic data sets, Ma-Hock et al. (2009) and Pauluhn (2010a). While not so problematic for determining a shape as data	**E1.** This EPA 2000 document is already cited as the external review draft in the CIB Reference section. A search of the EPA website did not locate the 2008 draft. However, the 2000 draft is available on the EPA website. **E2.** As the reviewer notes, the limitations in these data for BMD modeling are discussed in the CIB, as well as the statistical advantages	**E1.** The web address for the EPA 2000 document has been added to the reference citation. **E2.** In the revised CIB, the Ellinger-Ziegelbauer and Pauluhn [2009] and Porter et al. [2010] studies have been replaced with the

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Peer Reviewer 1 (cont.)	where no partial responses are shown, this feature creates a model that is heavily dependent on the scale and separation of the doses. Beyond the P-values, no diagnostics for the two fitted dichotomous models are provided. When one looks at the short-term studies, two of the three examined in Figure A-2 appear to have non-monotonic patterns, which are not easily captured by BMDS, the Benchmark Dose Software that was used. The continuous data and the categorized data are even more at issue. Therefore it is not appropriate to use Bench Mark Dose Modeling on any of the studies NIOSH analyzed in the CIB. Instead, we recommend that the NOAEL be used if comparisons between studies are needed, and for deriving OELs for the CIB.	of BMD modeling when feasible (Section A.2). Goodness of fit tests were performed based on the EPA BMD software, and only models that provided adequate fit to the data were included. Further statistical evaluation showed non-unique parameter solutions for models other than the multistage fit to the subchronic data (Section A.2.3.3). Concerning the short-term studies (Figure A-2), we agree that the data from Ellinger-Ziegelbauer and Pauluhn [2009] and Porter et al. [2010] studies were of equivocal fit to the minimum data criteria for BMD analysis, and these have been	more recent studies from the same laboratory on the same CNT material—Pauluhn [2010a] and Mercer et al. [2011]. The NOAEL and LOAEL estimates reported in the subchronic studies have been used (in addition to the BMD and BMDL estimates) to calculate the human equivalent working lifetime 8-hr TWA concentrations (Section A.6.2). The LOAEL and NOAEL estimates were shown to be similar to the BMD and BMDL estimates (Table A-12) and thus had little effect on the OEL derivation.

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Peer Reviewer 1 (cont.)		replaced by the more recent studies from these laboratories. Concerning the NOAEL, we agree that this effect level provides additional useful information and have added these analyses for the subchronic studies.	
	F. Appropriate considerations for the POD:		
	F1. The POD is the statistical estimate of the NOAEL, the place where the curve appears to be zero or a reflection of the study's resolution, not the BMCx or BMCLx. That is, the BMCx is an estimate of a point on the fitted curve, where the curve was fitted to observed incidence. It is the interpretation of the use of that point that is important. Thus, NIOSH correctly chooses, in general, to use BMD modeling rather than NOAEL estimation, *per se*, as a basis for its assessment, when appropriate data are available. One should match the POD to the capacity of the experiment and the endpoint of interest, and use it accordingly. The application of BMD analysis to	**F1.** According to EPA [2000], "The POD for BMD modeling is the BMDL, or the lower 95% bound on the dose/exposure associated with the benchmark response, typically 10% above the control response." NIOSH has used the 10% excess risk level in the absence of data suggesting otherwise.	**F1.** A reference citation to EPA [2000] has been added to a similar description of the POD in Section A.2.3).

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Peer Reviewer 1 (cont.)	derive 10%, 1%, and 0.1% excess risk levels is an incorrect use of the BMD methodology. Typically, the POD is taken by EPA as the 10% excess risk, but, depending on the endpoint and its background rate, it may be appropriate to choose a different POD. For example, a suitable POD is about 20% for the functional observational neurotoxicity battery because of a higher background. As another example, the AEGL Program chooses the lower of a BMC of 1% (BMC1) or a BMCL of 5% (BMCL5), looking at lethality (where controls survive at 100%). The AEGL program interprets the POD that it uses as being an estimate of the highest dose where the incidence of adverse effect is not statistically different from zero, based on the fitted dose-response curve. (That isn't an estimate of zero response as such; it's an estimate of the greatest dose where that occurs.)		
	F2. In Table A-6 NIOSH appears to intend to show how excess risk is associated with exposure, by tabulating the calculated human working lifetime airborne concentrations associated with several BMCx and BMCLx. The obstacle in using Table A-6 for this purpose, however, is the lack of a prior statement of the studies' resolution of each of the endpoints used. If, in fact, a suitable POD for both granulomatous inflammation and focal septal thickening is assumed to be BMC(L)10,	**F2.** Standard risk assessment approaches for non-cancer endpoints have typically assumed a threshold model, with extrapolation beyond the point of departure based on uncertainty factors, e.g., EPA	**F2.** Table A-6 (with 10%, 1%, and 0.1% excess risk estimates) has been omitted. The 10% risk estimates are already included in Table A-5. The 1%, and 0.1% excess risk estimates have been added in

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Peer Reviewer 1 (cont.)	then the three pairs of columns displayed in the table do not convey useful information regarding the workplace to the reader because they are below the level of resolution and neither the displayed BMC(L)1 nor the displayed BMC(L)0.1 is different from control (or zero). That is, there is no point in displaying them. If, however, the POD for these endpoints had been identified as the BMC(L)0.1 (the *lowest* of the three choices), then all three pairs would have been meaningful and indicate a range of doses throughout which the workplace exposures could be improved. This choice has not been set out by NIOSH in advance, with consideration of the data; thus, the three columns should not be displayed. Which response level is suitable to choose for the POD would depend on information about the characteristics of the endpoints (but the POD is rarely below 10% response with animal data).	[1994]. NRC [2009] and others have recommended using risk-based low-dose extrapolation for non-cancer endpoints. NIOSH practice has also included risk-based low-dose extrapolation for non-cancer endpoints. Thus, the 1% and 0.1% excess risk estimates have been retained in the CIB, but the uncertainties in these estimates have been clarified. In the absence of information about the shape of the dose-response relationship, a linear extrapolation would be most protective (i.e., unlikely to underestimate the risk). However, the actual risk may be much lower, including zero. This information	the text (Section A.3.3) along with discussion about standard low dose extrapolation approaches and uncertainties in the shape of the dose-response relationship for these early-stage noncancerous lung responses. Tables A-7 and A-8 have been added in the revised CIB to provide working lifetime excess risk estimates at various possible LOQ values of NIOSH method 5040. These excess risk estimates are near or above 10%, and do not require extrapolation far beyond the range of data.

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Peer Reviewer 1 (cont.)		has been added to the text (e.g., Section A.3.3).	
	G. In this CIB, in fact, the data themselves do not appear to support any modeling. Consider two instances illustrating this point in the CIB:		
	G1. Figure A-2 Ellinger… graph. This data set has 3 points. The control has a response, the lowest dose has a 0 response and the high dose has a response set at 1. Essentially there is a 0% response and a 100% response. Since a graph can be plotted it seems possible to put this data set into a BMD model and obtain a response. It is problematic to model this, however, because there are no intermediate data points to give one an assessment of the shape of the curve. This data set reflects a study "with only a single dose showing a response different from controls [which] may not be appropriate form BMD analysis" (Benchmark Dose Technical Guidance Document, 2000, §II.A.1.a.).	**G1.** In the revised CIB, the Ellinger-Ziegelbauer and Pauluhn [2009] is used only in the evaluation of dose rate (Section A.2.1.2).	**G1.** The Ellinger-Ziegelbauer and Pauluhn [2009] study has been replaced with the subsequent subchronic inhalation study on the same CNT material from the same laboratory [Pauluhn 2010a] (which is also included in the external review draft).

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Peer Reviewer 1 (cont.)	**G2.** Figure A-2 Porter… graph. This data set has 3 points: a control with 0% response and two data points at 20 and 80 units of exposure with essentially the same response (i.e., a plateau). This data set cannot be used to do any assessment of a non- or minimal response. All one can say is that the NOAEL is under 20 units of exposure. It typifies the "data set in which all non-control doses have essentially the same response level" described as falling short of the "Minimum data set for calculating a BMD" in the Benchmark Dose Technical Guidance Document (§II.A.4.). These two instances illustrate that these endpoints do not sustain the choices of POD made in the CIB. Thus, again (as in item 4), it is not possible to use Benchmark Dose modeling to get an OEL.	**G2.** Agree that the Porter et al. [2010] data are of equivocal value for the BMD modeling and that a subsequent study [Mercer et al. 2011] provides dose-response data which avoid these issues..	**G2.** The Porter et al. [2010] study has been replaced with a subsequent study on the same CNT from the same laboratory [Mercer et al. 2011] which provides a quantitative (continuous) measure of alveolar septal thickening.
	H. The CIB should review Pauluhn (2010b) which derived a different OEL for MWCNTs based on the same data used in the CIB.		
	H1. Pauluhn (2010b) arrives at a different OEL for Baytubes due to different assumptions, data, and calculations. Given this OEL is considerably higher than that derived by NIOSH using the same	**H1.** Agree that this provides a useful comparison of methods and	**H1.** A detailed evaluation of these different methods and assumptions has been

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Peer Reviewer 1 (cont.)	data – 0.05 mg Baytubes/m3 as a time weighted average – a discussion of these different approaches, and why the CIB value is more appropriate, should be offered.	evaluation of the influence of different assumptions on a health-based OEL.	added (Section A.6.3).
	I. Was the dichotomization of fibrotic effects done appropriately?		
	I1. At the 3 Feb. NIOSH public meeting, Juergen Pauluhn (author of one of the two key studies that form the basis of the OEL in the CIB) noted that the lowest dose where fibrotic effects were seen histologically may not represent irreversible fibrotic lesions (graded as a 1). Therefore, his suggestion was to use the data where a score of 2 was determined. This seems plausible, if the histopathology ranking system in his 2010 publication (and in that of Ma-Hock 2009) is unclear and if the CIB is to be based on irreversible adverse lung effects.	**I1.** Risk estimates for histopathology grade 2 or higher have already been provided (Table A-7 in the external review draft CIB, which is Table A-6 in the revised CIB).	**I1.** The description of the histopathology findings has been revised and clarified (Section A.2.1.3 and throughout the document) with regard to alveolar septal thickening and fibrosis.
	I2. While the discussion immediately above provides a biological argument against the choice of cut point selected by NIOSH, there is also a statistical argument against grouping the response severities as done by NIOSH. The CIB infers that	**I2.** These arguments are inconsistent with the data: First, the response proportion was zero in the	**I2.** Section A.6.2 and Table A-12 were added in the revised CIB to provide expanded discussion

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Peer Reviewer 1 (cont.)	use of histopathology grade 1 or higher provides a more sensitive response than grade 2 or higher (page 111 of the CIB). While the BMD(L)s are so much lower for the former, in this case that reflects a diminished ability to distinguish a dose response using this endpoint, not an increased sensitivity of the response. By definition, at any given dose, there will be more animals counted when all grades ≥1 are included than when all grades ≥ 2 are included. That is, when one includes any animal with a response at grade 1 or higher, one is including more affected animals in every group and reducing the ability to distinguish between treated groups; additionally, in the control group, by chance animals may be at grade 1 more readily than at grade 2, thereby diminishing discrimination between that group and the treated ones. Thus, in order to be able to discriminate and use the resulting dichotomized response to identify a dose response, a cut point of grade 2 should be used.	unexposed (control) and the lowest (0.1 mg/m^3) dose groups; thus there was no difficulty distinguishing the treated and untreated groups at category 1. Second, the BMDL estimate for category 2 (0.45 mg/m^3) is identical to the LOAEL reported in Pauluhn [2010a] (Table A-12), indicating that category 2, as the critical effect level, is a less sensitive and less protective endpoint to extrapolate to humans.	and comparison of possible critical effect levels. Also, Table A-6 (formerly Table A-7) provides the excess risk estimates for category 2 (which the reviewer apparently had not noticed).

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Peer Reviewer 1 (cont.)	**J. The OEL estimates for CNTs and CNFs should be supported by a clear statement of the Mode(s) of Action addressed by the studies used, and covered in the subsequent OEL.**		
	J1. Carbon nanotubes are thought to cause adverse lung effects through at least two different mechanisms: outcomes resulting from their behavior as poorly soluble particulates (due to the agglomerated nature of some MWCNTs), and behavior as singlet fibers. The data that are relied on principally in generating the OEL estimates in the CIB are from two subchronic studies that use agglomerated MWCNTs. It would be helpful to have a discussion of the postulated MOA, and associated resultant uncertainties, that underpin the CIB OEL values. Similar approaches are taken, for example, in the recent RfC document on ceria published by the US EPA (USEPA, 2009; see in particular the section on MOA beginning on page 46 of this IRIS assessment). This would be particularly helpful if any BMD modeling approach is reconsidered in issuing the final CIB.	**J1.** Agree that the mode of action evidence should be provided to the extent available. This information is included in Section 3, and Appendix Section A.2 with respect to the observed effects in animals and cells in vitro. We also agree it would be helpful to add a brief summary of the hypotheses and available evidence in the risk assessment section (Appendix A).	**J1.** Additional discussion on mode of action evidence has been added in the introduction to the risk assessment (Section A.1.1) and in a new section on sensitivity analyses of the alternative methods and assumptions and the associated uncertainties that pertains to a health-based OEL (Sections A.6.3.2. and A.6.3.2.1).
	J2. Please clarify the assumptions in the last paragraph on page 115: Does this paragraph assume that Haber's Rule applies to CNTs? Currently the data appear insufficient to predict the relationship one might see with CNTS and	**J2.** This paragraph discusses a comparison of the dose-response data from the 1-day and	**J2.** Expanded and clarified discussion of exposure metrics as relates to mode of action including

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Peer Reviewer 1 (cont.)	Haber's Rule cannot be inferred. More intermediate data points are required from the shorter-term studies, as well as data points derived from experiments with exposure durations greater than 90 days. The research and information needs noted on pages 59 – 61 are appropriate. In particular, the need for better quantification of worker airborne exposures to CNTs and CNFs, the conducting of chronic animal studies on CNTs, and the comparisons of CNT material used in animal studies with the CNTs found in the workplace air would be particularly helpful.	13-week inhalation studies in rats [Ellinger-Ziegelbauer and Pauluhn 2009; Pauluhn 2010a], with responses examined in each study at the same time point (13 weeks after the first exposure day). The analysis showed a consistent dose-response relationship for the data in both studies despite the difference in dose-rate (Figure A-4). These data could be considered consistent with "Haber's rule" although additional study is needed including cumulative exposure data as mentioned in CIB.	cumulative exposure (Section A.6.3.2.2). Added information on other standard risk assessment practices. For example, EPA [1994] states that to derive exposure limits (e.g., RfCs), "...cumulative exposure or time-weighted averages are appropriate for substances with long half-lives."

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Peer Reviewer 2	The draft NIOSH Current Intelligence Bulletin (CIB) "Occupational Exposure to Carbon Nanotubes and Nanofibers" represents a carefully considered and comprehensive assessment of the state of knowledge on occupational health risks associated with airborne carbon nanotube exposure, and draws well-reasoned conclusions on actions toward reducing health risks associated with exposure. The document responds to both growing awareness of the potential risks associated with carbon nanotube exposure, and increasing use of carbon nanotubes in commercial products. In doing so, it addresses a number of issues that are important to the safe and successful handling and use of carbon nanotubes in workplaces, and does so in a timely manner. I would like to commend NIOSH for undertaking this review and assessment. Developing clearer guidelines on the safe handling of carbon nanotubes is critical to their long-term safe, sustainable and successful use. In drafting this document, NIOSH had taken an important lead in beginning to establish such guidelines. However, given the tremendous uncertainty over the physical and chemical nature of carbon nanotubes, the hazards that different types of carbon nanotubes present, the nature of occupational exposures, the validity and interpretation of *in vivo* toxicity studies and the meaning of derived dose-response		

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Peer Reviewer 2 (cont.)	relationships, this must be viewed as just being the beginning of a process. **A1.** In the draft CIB, NIOSH takes the pragmatic step of treating all carbon nanotubes and nanofibers as nominally the same material – whether they are single walled, multiple walled, functionalized, long, short, straight, curved, tangled, agglomerated, having many un-terminated graphene edges or just a few, etc. From a mechanistic perspective, this is hard to justify – while the biological relevance of the specific chemistry and morphology of different carbon nanotubes (including nanofibers) is far from clear, there is strong evidence that chemistry and morphology together have a profound influence over biological interactions and toxicity. Having said this, there is some merit in taking a crude initial stab at establishing exposure limits based on the material family rather than specific components *in the absence of further information.* This is an approach that allows gross common behavior to be captured in a single and implementable exposure level, and provides a route to at least reducing the potential for harm to occur. However, it should be clearly recognized that the approach is a pragmatic compromise, and one that should be revisited and revised on a regular basis. In particular, there is increasing evidence that the mode of action associated with compact or short and straight and	**A1.** NIOSH researchers are aware of the variety and complexity of CNT/CNF particles and investigating a TEM method to categorize and quantify the different structures. NIOSH is disseminating its findings through conferences, journal publications and its website and will continue to do so as additional information becomes available. Section 3 *Evidence of Potential Adverse Health Effects* describes the physical characteristics of the CNT and CNF exposures administered in the	**A1.** Section 3 *Evidence of Potential Adverse Health Effects* has been revised to provide additional clarity on the interpretation of the available toxicology evidence. Section 4 *Conclusions - Hazard and Exposure Assessment* has been revised to describe what mechanistic information is available and what research is needed to provide better risk management recommendations.

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Peer Reviewer 2 (cont.)	long carbon nanotubes is markedly different – the latter being more closely associated with carcinogenic potential – and this should ideally be reflected in subsequent risk assessments and recommendations for reducing risk.[1] As an associated point, there is considerable lack of clarity in the document concerning the physical nature of carbon nanotubes associated with inhalation exposure. Throughout the document, there is an implication that these are fiber-like entities. However, relatively few carbon nanotube materials conform to most people's understanding of "fiber-like". Specifically, many multi-walled carbon nanotube materials consist of relatively short nanotubes, while some consist of nanotubes that are millimeters to centimeters long; single walled carbon nanotube materials typically have a complex and convoluted morphology, which does not conform to the idea of a straight, isolated fiber; some unprocessed carbon nanotube materials contain appreciable amounts of non-tubular elemental carbon; most carbon nanotube materials	animal studies and provides pertinent conclusions where appropriate as to the relationship of the exposure characteristics to the observed health effect. Section 4 *Conclusions – Hazard and Exposure Assessment* describes what is known about the relationship of the physical and chemical properties of CNT and CNF and the observed health outcomes in animals. The CIB notes that additional information is needed to understand the mechanisms that cause	

[1] Donaldson et al. have suggested that carbon nanotube materials demonstrate particle-like or fiber-like behavior, depending on their physical form (Donaldson, K., R. Aitken, L. Tran, V. Stone, R. Duffin, G. Forrest and A. Alexander (2006). "Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety." Toxicological Sciences 92(1): 5-22.). This hypothesis has been supported by a number of studies, including the work of Poland et al. on the response to long and short multi-walled carbon nanotubes introduced into the abdominal cavity of mice (Poland, C. A., R. Duffin, I. Kinloch, A. Maynard, W. A. H. Wallace, A. Seaton, V. Stone, S. Brown, W. MacNee and K. Donaldson (2008). "Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study." Nature Nanotechnology 3: 423-428.)

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Peer Reviewer 2 (cont.)	exist in an aggregated state, where the size and morphology of the aggregates is dependent on handling and – in exposure studies – the mechanism of aerosolization. Given that morphology is likely to play some role in determining exposure and hazard for these materials, at the very least it would be helpful if the draft CIB could document – ideally using Transmission Electron Microscope images – the range of morphologies potentially encountered.	adverse respiratory effects.	
	A2. The Current Intelligence Bulletin also takes the pragmatic approach of recommending a Recommended Exposure Limit (REL) based on respirable mass concentration. Despite the substantial uncertainties over modes of action that raise questions over relevant dose metrics, this would appear to be a sensible and justifiable decision. Current evidence suggests that it is the alveolar region of the lungs that is predominantly susceptible to inhaled carbon nanotube material, supporting the decision to focus in respirable nanotubes. And while there remains uncertainty over whether response is most closely associated with the number, morphology, surface area, mass etc. of deposited fibers or particles, mass concentration is indicated to be a crude but effective indicator of effect in most studies to date. Thus while evidence may well arise suggesting alternative dose metrics in the future, respirable	**A2.** Agree that these are relevant discussion points concerning the mode of action and dose metric evidence and uncertainties.	**A2.** Additional discussion of the mode current evidence and uncertainties is provided in Sections 5.3, A.1.1, A.6.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)	mass concentration is a reasonable starting point. **Question:** **B. Is the hazard identification and discussion of health effects for CNT and CNF a full and reasonable reflection of the animal studies and other scientific evidence in the scientific literature?** **B1.** The draft CIB presents a comprehensive review of the published scientific evidence on the potential hazards associated with carbon nanotube and nanofiber inhalation. The key studies are identified and, where deemed appropriate, incorporated into the risk assessment. However, the draft CIB as it stands has two limitations in particular in this: There is a paucity of critical evaluation of the validity and robustness of studies, and there is a marked lack of differentiation between effects associated with particle-like behavior, and effects associated with fiber-like behavior. On the first limitation, there is still a considerable lack of expertise and "art" in conducting well	**B1.** The finding of similar BMD(L) estimates across the various study designs and types of CNT suggests that these risk estimates are robust for the noncancerous lung effects, given the methods and models used in this risk assessment. Some of the variability in the risk estimates across CNT	**B1.** Additional evaluation of the study data quality is provided in Section A.4.5. Also provided additional analysis and discussion of the sensitivities and uncertainties in the risk estimates (Sections A.6 and 5.3).

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)	characterized, interpretable and repeatable inhalation studies with carbon nanotubes. There is uncertainty over how generation and delivery methods alter the physicochemical nature of the material and how this impacts on exposure, deposition and response; there is uncertainty over which material attributes to characterize in studies, and how to appropriately quantify them; and there is uncertainty over the identification and interpretation of endpoints. As a consequence, the validity and comparability of many published studies needs to be approached with some caution – especially if they are to be used as the basis of a quantitative risk assessment. The draft CIB would benefit from a more robust discussion of the limitations and quality of the studies used.	studies could be due to sources of experimental variation such as these. Despite this variability, the BMC(L) estimates (up to approximately two orders of magnitude), these working lifetime 8-hr TWA concentrations were all relatively low mass concentrations relative to OELs for other poorly soluble particles.	
	B2. On the second limitation, Donaldson et al.[2] have proposed that different forms of carbon nanotube material demonstrate markedly different modes of action – with compact materials predominantly showing insoluble particle-like behavior in the lungs, and long, thin fiber-like materials demonstrating a biological behavior that conforms to the fiber paradigm. The potential for	**B2.** Limitations in exposure metrics other than mass preclude a risk assessment and OEL based on specific CNT structures.	**B2.** Concerning potential cancer effects of CNT, the most current studies on genotoxicity and carcinogenicity of CNT have been added to the revised CIB.

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

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)	such markedly different behavior – together with the increased likelihood for carcinogenesis in the latter case – suggests that additional thought should be given to treating all nanotube materials as having the same mode of action.		Although quantitative dose-response data are not available to estimate the potential cancer risk from inhalation of various types of CNT, these recent studies have been included in the hazard assessment, which is part of the evidence considered in deriving the REL.
	Question: **C. Is the risk assessment and dosimetric modeling methods used in this document appropriate and relevant?**		
	C1. The risk assessment and dosimetric modeling methodologies used in the draft CIB are in line with conventional practice. However, I do have concerns over the robustness of the assessment, given uncertainty over the quality of the data and how sparse the data are in many cases around the Benchmark Dose (BMD). While the modeling approach adopted is reasonable, I do have concerns	**C1.** Agree that additional analysis of uncertainty in risk assessment would be useful, including comparison of BMD(L) estimates to the NOAEL and	**C1.** Added a detailed sensitivity analysis of the methods and assumptions used in the risk assessment and the impact on the derivation of a health-based OEL (Section

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)	that the derivation of quantitative BMDL values in appendix A look qualitatively as if they are likely to be highly dependent on the models used and the uncertainty in the fitted data. In this regard, I would like to see a sensitivity analysis where possible, and indications of confidence or error on the derived values of BMD, BMDL, BMC and BMCL. **Question:** **D. Is the use of respirable mass as a dose metric appropriate for estimating worker risks from inhalation to CNT and CNF?** It is my opinion that the use of respirable mass as a dose metric is appropriate. While alternative metrics may be justified on mechanistic grounds (although the state of the science is not sufficiently advanced to indicate which alternative metrics would be more appropriate), current indications are that mass concentration in the respirable size range is an adequate indicator of potential risk. **D1.** That said, the potential for fiber-like behavior does raise the question of whether some forms of carbon nanotubes and nanofibers should be evaluated on a number concentration basis. There is some justification for this where the material is	LOAELs in the subchronic studies. **D1.** NIOSH researchers appreciate the complexity and variety of CNT/CNF materials, including	A.6). The major and minor factors contributing to uncertainty in the risk estimates are discussed in Section 5.3. **D1.** Additional information is provided in Section 4 *Conclusions – Hazard and Exposure*

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)	comprised of long, straight fibers, where these fibers are unbound, and where agglomeration is relatively low. However, for materials where the fibers are short, where they are highly agglomerated, where they are encapsulated in another material (in the particulate form), where they are tightly entangled, and where they have complex morphologies number concentration is not indicated as being a useful exposure metric. This holds in particular for single walled carbon nanotubes, which do not exhibit a fiber-like morphology in a conventional understanding of the term.	their composite dusts. In addition to NIOSH 5040, TEM is being applied to determine the number concentrations of CNT/CNF particles *classified according to morphology and size* rather than just total particle counts. However, a TEM-based method for counting the many different structures is nontrivial and has not been validated. Further, the relative toxicities of the different structures are not yet clear. Classification of the structures will contribute data for future studies of this issue.	*Assessment* describing current knowledge on what physical and chemical properties appear to be associated with observed lung fibrosis in animals. Based on current animal data the only dose-response information is associated with the respirable mass of CNT or CNF.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)	**Question:** **E. Are the sampling and analytical methods adequate to measure worker exposure to carbon nanotubes and nanofibers?** **E1.** The proposed NIOSH Method 5040 has merit for measuring exposure to carbon nanotube material. However, the draft CIB has negligible information demonstrating the applicability of the technique to these materials. Given the unique nature of carbon nanotubes, I do not think it is sufficient to state that the method works for elemental carbon, with the implicit assumption that it will also work for carbon nanotubes and nanofibers. **E2.** As discussed above, there is also a question of background interference from other workplace sources of EC. Given the apparent lack of validation of the method and the uncertainty associated with interference from other sources of EC, it would be helpful if the draft CIB discussed the limitations of the method in more depth, and suggested alternative or complimentary monitoring methodologies – such as the parallel use of Transmission Electron Microscopy sampling and analysis.	**E. Response to comments E1 and E2 on the appropriateness of analytical methods:** Multiple techniques are needed to characterize exposure to CNT/CNF, and NIOSH investigators have applied multiple techniques in all field studies, the most comprehensive of which was a study at a CNF manufacturing facility [Birch et al. 2011b, Birch 2011a, Evans et al. 2010]. See above response to Reviewer 1.	**E. Response to comments E1 and E2:** Additional details about the sampling and analysis of CNT and CNF are provided in Section 6.1 *Exposure Assessment* and Appendix C. Guidance is given on the use of other analytical methods (e.g., electron microscopy) for the characterization of CNT and CNF.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)	Given the uncertainty over the general applicability of Method 5040 on its own, it may be difficult to support the recommendation of a 7 $\mu g/m^3$ REL, as it is entirely based on this exposure characterization method.	Mass concentration is a traditional exposure metric, but other metrics have been proposed as being more relevant to nanomaterials, including particle number and surface area. These alternative metrics may have relevance for some materials in controlled atmospheres, such as in animal inhalation studies, but they are neither selective nor quantitative, and the available instruments for their measurement in the field are area monitors. These issues present practical limitations on the metrics applied to worker exposure assessment. In previous CNT/CNF studies by NIOSH	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		researchers [Birch et al. 2011b, Birch 2011a, Evans et al. 2010, Methner et al. 2010a, b; Dahm et al. 2011], direct reading instruments for particle size, number, and 'surface area' were useful as indicators of background and byproduct aerosols but not CNT/CNF monitoring. Multiple metrics are being applied to ongoing NIOSH surveillance studies at primary manufacturer and secondary user sites. The purpose of this research is to characterize emissions, with a goal of collecting health relevant exposure data. NIOSH appreciates that some facilities may not have adequate	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		resources for extensive monitoring. NIOSH is actively recruiting companies to participate in its surveillance studies and can provide comprehensive workplace assessments in such cases. However, some companies may prefer to conduct monitoring in-house and seek practical monitoring guidance. In this regard, NIOSH 5040 should provide a useful estimate of exposure to CNT/CNF when these materials are the main source of EC. As discussed in the CIB, a bulk sample of the CNT/CNF should be analyzed whenever possible to establish the thermal profile for the material(s) and rule out any potential	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		problems in the analysis. A bulk sample also can be used to determine other material properties, such as metal content by inductively coupled plasma (ICP) with detection by atomic emission spectroscopy (AES) or mass spectrometry (MS). Use of a metal catalyst as a surrogate measure of CNT/CNF has been suggested previously and was considered by NIOSH researchers, but this approach has limitations. Namely, lack of correlation with the CNT/CNF concentration and inadequate detection limits. Iron was not a useful indicator of CNF exposure in a CNF manufacturing	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		facility. The iron and CNF concentrations were not correlated because the major iron source was not CNF derived. Further, even if a metal is a selective marker of CNT/CNF exposure, the detection limits for ICP/AES will likely not be adequate for quantification at low CNT/CNF concentrations (e.g., the NIOSH REL) due to the low metal contents (e.g., typically ≤ 1%) of current products. Currently, a draft TEM-based method for quantitative measurement of CNT/CNF 'structures' is being investigated by NIOSH, but the problem of categorizing the many	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		types of structures has not been adequately addressed. Further, even if the different types of structures can be consistently sorted (by different analysts), there currently is no basis (e.g., aspect ratio restriction for asbestos fibers) for weighting their potential toxicity. The recommendation of NIOSH 5040 is based on field studies and laboratory data. In 2011, two papers on its application to CNT/CNF field studies were published [Birch et al. 2011b; Dahm et al., 2011]. A paper on its application to a variety of CNT/CNF materials is in preparation. Several potential issues with Method 5040 are discussed in the CIB:	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		1) early loss of EC (in helium), 2) need for extended analysis time for some materials (e.g., highly graphitized), 3) a manual OC-EC split in some cases, 4) inability to distinguish between free CNT/CNF and CNT/CNF bound in a polymer composite matrix (and possible OC overloading by polymer, and 5) environmental background EC. Background EC from various sources such as diesel engines limits the ability to make low-level measurements. Initial workplace assessments must carefully consider background measurements and potential interferences to determine whether	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		these issues apply in a given workplace. The issue of background interferences is not new. It was discussed in depth when NIOSH 5040 was proposed for workplace monitoring of diesel particulate matter (DPM). Sources of EC background are discussed in the CIB. As stated above, NIOSH 5040 should provide a reasonable estimate of workers' exposure to CNT and CNF at the NIOSH REL of 1 $\mu g/m^3$ 8-hr TWA when the predominant workplace exposure to EC material is CNT or CNF. As with all analytical methods, the LOD is a varying number. However, the EC LOD originally reported for NIOSH	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		Method 5040 was about 2 $\mu g/m^3$ or an LOQ of 7 $\mu g/m^3$, as a worst-case value [NIOSH 2010]. It was based on analysis of pre-cleaned media blanks from different filter lots, over a six month period, and by different analysts at two different laboratories. Further, variability for the total carbon (TC) results was used to estimate the LOD rather than EC results. These combined factors gave a very conservative (high) estimate of the EC LOD. In practice, a much lower EC LOD is obtained by NIOSH 5040 because the variability for EC results for a set of media blanks submitted (with the sample set) for the LOD (LOQ) determination is	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		much lower than that for the TC results. See CIB for further discussion.	
	Question: **F. Are there additional relevant studies or methods that NIOSH should consider in developing the REL for CNT and CNF?**		
	F1. I do not think there are any other studies at present that would change substantially the conclusions and recommendations of the draft CIB. Regarding methods, there has been some suggestion of using metal contaminants as markers for carbon nanotubes, as used by Maynard et al. (2004)[3]. This is an approach that is applicable where the material in question has a clear and consistent fingerprint. But it runs into difficulties where there is wide variation in contaminant levels between processes, or within processes – either as a product is successively processed, or through batch-to-batch variation. There are also some carbon nanotube production processes that result in negligible metal contamination.	**F1.** Iron was not a useful indicator of exposure in a CNF manufacturing facility. Because the major iron source was not CNF derived, there was no correlation between the iron and CNF concentrations. Even if a metal was a selective marker of CNF exposure, the LOD for ICP/AES would likely not be adequate to use a	**F1.** Section 6.1 *Exposure Assessment* and Appendix C describes how to optimize the analysis of CNT and CNF samples using Method 5040.

[3] Maynard, A. D., P. A. Baron, M. Foley, A. A. Shvedova, E. R. Kisin and V. Castranova (2004). "Exposure to Carbon Nanotube Material: Aerosol Release during the Handling of Unrefined Single Walled Carbon Nanotube Material." J. Toxicol. Environ. Health **67**(1): 87-107.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 2 (cont.)		metal as a surrogate for CNT/CNF at low concentrations (e.g., near the EC LOQ). If a catalyst metal is used as a surrogate measure of CNT/CNF, correlation with the CNT/CNF concentration (mass or otherwise) and adequate detection limits are required. See previous response above on methods and metrics and their associated limitations.	

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 3	This document presents one of the first credible attempts to provide an evidence based exposure limit for carbon nanotubes. This is a difficult and challenging task given the many variations of carbon nanotubes which have been described in the literature, the limited evidence available in relation to potential exposure for these types of materials, the lack of any agreed measurement methods for estimating exposure to these materials, the limited information available in relation to the hazardous nature of these materials, and widely described issues in the literature relating to the appropriate choice of metric by which exposure to these materials should be addressed (accessed). However, in a general sense the document is well balanced, proportionate and pragmatic document which does draw together the key and important elements of the evidence across the range of the risk issues associated with potential exposure to CNTs. In relation to the exposure situations described, and the health effects used as the basis of the derivation of the limit, NIOSH have identified all of the appropriate and relevant studies which could be used to come to the conclusions that they have come to. It has been widely discussed in the literature, the potential similarities between some types of carbon nanotubes and asbestos. The similarities based on what is known as the "fibre paradigm". That is that		

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 3 (cont.)	long durable bio-persistent fibres, such as asbestos, if inhaled, have the potential to enter the plural space in which they are retained and in due course can give rise in the development of mesothelioma. Elements of the fibre paradigm have been demonstrated with some types of carbon nanotubes. For example Poland *et al* (2008) have shown a length dependent effect associated with the development of inflammation for carbon nanotubes injected directly into the peritoneal cavity of a mouse. Osmond *et al* (in press) have compared the durability of CNT as compared with asbestos fibres. Many other papers and reports have speculated on the potential association (e.g. Maynard *et al* 2006). **A1.** It appears that NIOSH have not considered this potential health effect in deriving their exposure limits. Rather they have focused on the health effects of pulmonary fibrous and granulomatous inflammation. To some extent this is justified. These effects (as described in the quoted studies in the document) are ones for which inhalation studies are available which provide the basis for establishment of a dose response relationship and therefore the establishment of an occupational exposure limit. Whilst they have generalised these studies for all carbon nanotube types (including single and multiple for example) it is recognised that only some (perhaps limited	**A1.** NIOSH acknowledges the uncertainties in the mechanisms in the biological responses to CNT and CNF, and recommends precautionary measures to reduce the risk of occupational lung diseases in workers with potential exposures to CNT and	**A1.** Revisions were made to the CIB to clarify that the quantitative risk assessment is based on the noncancerous lung effects (Executive summary and Appendix A introduction).

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 3 (cont.)	number) of carbon nanotube types are actually likely to or provide the possibility of generating aerosol releases that may be considered to be fibres (according to the WHO definition). It therefore makes some sense to develop a limit based on the evidence which is available, rather than for a small sub category of materials, for which there is not at all clear whether or not there will ever be exposure. However there are two dangers in this approach. Firstly, if carbon nanotubes can be released in a form that makes them consistent with long durable fibres such as those evaluated in the Poland study and if exposure to these occurs then it is highly likely that the recommended exposure limits produced by NIOSH will not be at all protective to those who are exposed at that level. To be clear, an exposure limit based on the fibre paradigm would result in a level that maybe several orders of magnitude below that currently being recommended by NIOSH. This clearly provides a cause for concern.	CNF (Executive Summary of external review draft and revised document).	
	B1. Given the knowledge and the prevalence of the discussions relating to this potential fibre paradigm issue for carbon nanotubes, and given that there is no clear statement within the current document that this is NOT the basis on which the limit has been developed, it is quite conceivable that people who use this document but who do not clearly read or carefully understand the basis for the derivation of	**B1.** Limitations in the CNT exposure measurement methods for metrics other than mass (e.g., no TEM-based exposure by CNT size analogous to asbestos), as well as	**B1.** The hazard assessment has been updated to include the most recent studies on the genotoxic and carcinogenic effects of CNT. Although these studies do not

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 3 (cont.)	the proposed limit will expect that the limit value produced will be protective for CNTs of the types which can be released as fibres. To some extent this issue could be resolved with some clear statements which indicated what health effect the limit is derived on but making specific reference to the fibre paradigm and indicating that this is NOT the basis for which the limit has been derived.	limited animal dose-response data (e.g., no data on cancer effects from CNT administered to the lungs) preclude a quantitative risk assessment of specific CNT structures and cancer risk or the development of an OEL based on air concentration of carcinogenic structures. Agreed that greater emphasis or clarification is needed that the risk assessment is based on the noncancerous lung effects and that there is uncertainty concerning the potential cancer risk at the REL for various types and structures of CNT and CNF.	provide a quantitative basis for the REL, they are considered in the overall evaluation of the health effects data and provide a basis for a higher level of precaution. The CIB has been revised to clarify that the quantitative risk assessment is based on the noncancerous lung effects and that the OEL may not be protective for either noncancerous or possible cancer effects (Executive summary; Section 5; Appendix A).

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 3 (cont.)	**C1.** NIOSH (on page 42) note that the REL that derived may not be completed health protective. In fact they indicate that the animal data-based risk estimates indicate that workers may have a greater than 10% excess risk of developing early stage pulmonary fibrous if exposed over a full working life time at this value. The value is chosen as it is the limit of quantitation (LOQ) of NIOSH method 5040 which is currently the recommended analytical method for measuring airborne CNT. I am not sufficiently familiar with NIOSH's approach in relation to these to say whether these are standard approach or not but I do not believe that greater than 10% excess risk is the normal criteria which NIOSH or indeed other limit setting organizations would choose. It would be very helpful that within this document a REL calculated according to the usual criteria was to be produced even if at the current time analytical methods were not available by which this could be measured. It could be further recognized more clearly that this proposed limit is only one based on analytical methods and that more data and indeed better methods are required in order to control exposure to a limit at which the excess risk is acceptable.	**C1.** See previous responses above. NIOSH researchers have applied multiple methods to characterize exposure to CNT/CNF, with each having the limitations noted previously. NIOSH continues to investigate alternative methods that may offer quantitative, selective, low-level measurement of CNT/CNF. Improvements in the analysis of CNT and CNF have lowered the limit of quantitation (LOQ) for Method 5040 to around 1 $\mu g/m^3$. NIOSH is recommending a REL of 1 $\mu g/m^3$. At the proposed REL some	**C1.** Section 6.1 *Exposure Assessment* and Appendix C provide guidance on optimizing the analysis of CNT and CNF. The NIOSH REL has been reduced to 1 $\mu g/m^3$ which decreases the residual risk for pulmonary fibrosis. Additional risk estimates have been provided at other LOQ values for NIOSH Method 5040 (Tables A-7 and A-8). In addition, other standard risk assessment methods have been evaluated (based on NOAEL approaches) to evaluate the influence of alternative methods and assumptions on the

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 3 (cont.)		residual risk of developing pulmonary fibrosis from exposure over a working lifetime still exists. Given the large number of individual CNT or CNF structures at a low mass concentration, a cancer risk may also exist, although the data are insufficient at this time evaluate the cancer risk in workers or develop quantitative risk estimates.	derivation of the REL (Section A.6.3).
	D1. Further discussions of this issue and the clear statement about the limitations of the methods would be beneficial, as would the encouragement to develop new protection methods which would allow detection at lower levels. Whether method 5040 will be the most appropriate, long term, remains to be seen.	**D1.** See responses above regarding methods and metrics and their associated limitations.	**D1.** Section 6.1 *Exposure Assessment* and Appendix C provide a thorough discussion of the limitations in using Method 5040 for CNT

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 3 (cont.)			and CNF determination. Guidance is provided on how to optimize sample collection and analysis.
	E1. In conclusion, NIOSH are to be congratulated for producing such a clear and well thought out document. My concern is, for the reasons described above, the limit value proposed will not be sufficiently protective for some types of CNT and will not prevent instances of disease in population which are exposed to carbon nanotubes.	**E1.** This concern is why NIOSH has recommended reducing exposures as low as feasible below the REL and has recommended as a priority research area the development of more sensitive and specific measurement methods.	**E1.** We have tried to clarify this message in the CIB.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4	**Question:** **A. Is the hazard identification and discussion of health effects for CNT and CNF a full and reasonable reflection of the animal studies and other scientific evidence in the scientific literature?** **A1.** The CIB provides a complete review of the available (as of mid-2010) peer-reviewed toxicological health effects data for single and multi-wall carbon nanotubes as well as carbon nanofibers. The interpretation and discussion of the study results, as well as the strengths and weaknesses of the various study methodologies, is appropriate. **Question:** **B. Is the risk assessment and dosimetric modeling methods used in this document appropriate and relevant?** **B1.** The risk assessment and dosimetric modeling methods utilized in the CIB represent the current	**A1.** Agree in part, although additional evaluation of the sensitivity and uncertainty in these methods was suggested by other reviewers. **B1.** Agree in general, although specific revisions have been	**A1.** Additional analyses of the sensitivity and uncertainty in the risk assessment methods and assumptions has been added (Section A.6). **B1.** Specific revisions as noted in these responses have been

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4 (cont.)	state-of-the-art for this type of application. The authors of the risk assessment have appropriately utilized a benchmark dose (BMD) approach to modeling the toxicological data from the relevant selected studies, and have appropriately noted the limitations of the available data for use in the applied BMD methodology. Primary emphasis should be placed on the risk assessment results calculated from the two sub-chronic inhalation studies (Ma-Hock et al. 2009, Pauluhn 2010) which are most relevant to the human route of exposure and exposure periodicity. The short-term instillation and aspiration studies provide information on potential hazard and mode of action, but are of limited utility for use in extrapolating human health risks.	proposed by other reviewers. Although the subchronic inhalation studies are generally considered to provide the best data for risk assessment, the short-term studies provide data for SWCNT and for other types of MWCNT, for which no subchronic inhalation studies were available. Moreover, the working lifetime 8-hr TWA concentration estimates derived from these short-term studies were consistent to the estimates from the subchronic studies.	made in Appendix A. Additional emphasis on the subchronic inhalation studies has been provided in Section A.6, in which the methods and assumptions in the risk assessment have been evaluated using these subchronic inhalation data.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4 (cont.)	**B2.** I concur with the thrust of the public comments on the CIB regarding the need for a sensitivity analysis that discusses which step(s) constitute the greatest source of uncertainty with respect to the multi-step methodology used to develop the risk assessment. Such an uncertainty analysis would provide the reader with a perspective on which of the numerous steps (and associated data selection and assumptions) of the risk assessment methodology are of greatest influence on the uncertainties associated with the final risk characterization. The uncertainty analysis would also be informative for indicating which aspects of the risk assessment would benefit greatest from investment in further research and data development. While a quantitative sensitivity analysis would be preferable, at a minimum a qualitative assessment of which components of the risk assessment present the largest sources of uncertainty should be included in the CIB. **Question:** **C. Is the use of respirable mass as a dose metric appropriate for estimating worker risks from inhalation to CNT and CNF?**	**B2.** Agree.	**B2.** Sections have been added to provide a detailed sensitivity analysis of the methods and assumptions used in the risk assessment (Section A.6) and an evaluation of the major and minor factors influencing the OEL derivation (Section 5.3). These new sections provide both qualitative and quantitative information on the uncertainty which is also relevant to assessing the research needs.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4 (cont.)	**C1.** Since mass-based dose (for the instillation and aspiration studies) or mass-based exposure (for the inhalation studies) was the only available consistent exposure metric reported in the animal studies upon which the estimated human health risks were based, respirable mass is the only currently available basis for extrapolation of the full body of animal study data in estimating worker risks. However, future animal and human studies will hopefully provide information on exposure metrics (e.g., tube or fiber number and size, surface area) that based on experience with other fibers such as asbestos as well as ultrafine particles are likely to prove more relevant to estimating worker risks than a mass-based metric. Therefore, the use of respirable mass as the basis for estimating worker risks should be revisited as part of an expedited review of the scientific literature on CNT/CNF to determine whether an update of the proposed recommended exposure limit (REL) is warranted. **Question:** **D. Are the sampling and analytical methods**	**C1.** Agree.	**C1.** Although already included in the CIB, additional emphasis has been given to the research need to develop more sensitive and specific measures of exposure to CNT and CNF.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4 (cont.)	**adequate to measure worker exposure to carbon nanotubes and nanofibers?** **D1.** The selection of a monitoring method that measures elemental carbon mass is not likely to provide information on the CNT/CNF exposure metric of greatest relevance to assessing human health risks. The rationale presented in the CIB for selection of NIOSH Method 5040 rests primarily on it being the pragmatic approach to a broad-scale CNT/CNF monitoring protocol due to current cost and technological feasibility considerations. However, I concur with the recommendation made in the comments of the Dutch Expert Committee on Occupational Safety that the CIB strengthen language that encourages the use of additional analytical techniques discussed in the CIB such as TEM for work environments with the highest likely exposure potential. The CIB would benefit from additional discussion of other potential sampling and analytical methods (e.g., TEM) that could be used to augment the NIOSH Method 5040 with regard to measuring worker exposure to CNT/CNF in potentially higher risk work environments. Expanded discussion in the CIB of the characteristics of potentially higher risk work	**D1.** NIOSH researchers have applied multiple exposure metrics, including the use of microscopy to characterize CNT/CNF materials (particle morphology, size, and composition) and quantify worker exposure. A microscopy method based on TEM analysis is currently being investigated but has not yet been validated. See above previous discussion on methods and associated limitations.	**D1.** A discussion of alternative exposure metrics for measuring CNT and CNF exposures is discussed in Section 6.1 *Exposure Assessment* and Appendix C.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4 (cont.)	environments would be beneficial to supplement a recommendation for additional monitoring protocols for such work environments. **Question:** **E. Are there additional relevant studies or methods that NIOSH should consider in developing the REL for CNT and CNF?** **E1.** As discussed above, the REL for CNT/CNF should include reference to use of a TEM monitoring protocol (e.g., NIOSH Method 7402) for work environments with the highest likely exposure potential.	**E1.** See response above regarding TEM and other methods and associated limitations (note: NIOSH 7402 does not give a direct measure of asbestos fiber counts; it is used to adjust the total fibers counted by PCM (Method 7400), based on the fraction of fibers confirmed as asbestos by using	**E1.** A discussion on the use of TEM is given in Section 6.1 *Exposure Assessment.* No data currently exist to quantify exposures to airborne CNT or CNF by tube count (tube/cm^3). Criteria have not yet been developed for the counting and sizing of tubes by electron microscopy nor does there exist any animal data that provides quantitative data to determine what physical

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4 (cont.)		TEM Method7402.	dimensions should be included in the criteria for the sizing and counting of tubes
	F. General Comments:		
	F1. The CIB notes that the proposed REL for CNT and CNF is based on the limit of quantification (LOQ) for the NIOSH Method 5040 rather than on a level of exposure that provides adequate worker protection from excess health risks (CIB pgs. 6-7). Further, the CIB acknowledges that current scientific evidence suggests that use of exposure metrics such as number concentration of defined CNT/CNF dimensions are likely a better predictor of adverse health effects such as lung fibrosis than the use of a mass-based exposure metric, and that NIOSH Method 5040 may not be sufficiently sensitive to fully capture CNT/CNF concentrations at low volume levels (CIB pg. 7). As noted in the review of occupational exposure limits (OELs) for nanomaterials by Schulte et al. (2010), the Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA) and the British Standards Institution (BSI) have proposed occupational exposure limits for carbon nanotubes and fibrous	**F1.** As explained in the CIB, the NIOSH REL initially proposed was based on an LOQ (for EC) that is much higher than normally obtained. In practice, an LOQ near 1 $\mu g/m^3$ (or lower) can be obtained. Mass is a traditional exposure metric, and risk estimates from the animal data are based on mass concentrations; however, mass may not be the most relevant metric. Though expensive and tedious, a TEM-based method may provide	**F1.** Section 4 *Conclusions-Hazard and Exposure Assessment* provides the scientific evidence used to support the development of a REL based on a respirable mass concentration. Section 5 *CNT Risk Assessment and Recommended Exposure Assessment* provides the modeling of the dose response relationship between the respirable mass of CNT and the development of pulmonary fibrosis observed in mice and rats.

Commenter	Summary of Comments Received	Response	Changes to CIB
Peer Reviewer 4 (cont.)	nanomaterials respectively of 0.01 f/cm^3. An assessment of the strengths and weaknesses of the IFA and BSI recommended OELs, and NIOSH's rationale for not adopting an REL consistent with that of the IFA/BSI OELs, would provide the reader with a useful comparison to the 7µg/m^3 REL proposal. The comments above should not be construed as opposing the adoption of the proposed REL of 7µg/m^3 as an interim recommended exposure limit that should be reviewed and if necessary updated as soon as possible to consider whether an REL based on an alternative exposure assessment approach that is likely to be more reflective of the potential human health risks, e.g., CNT/CNF number and size-based exposure metric, should be adopted. Such an approach would encourage the monitoring technology industry to invest in the development of reasonable cost equipment for such measurement approaches with the understanding that a substantial market will develop for assessments of these metrics.	more relevant data if it is shown that a structure (tube) count exposure metric best reflects the toxicological evidence. NIOSH is conducting studies to better understand the mechanisms causing adverse respiratory effects in exposed animals. The main problem with a count-based method is the large variety of possible CNT/CNF structures, occurring as entangled networks, bundles, agglomerates, etc. rather than discrete fibers, as with asbestos. This complicates the counting process with respect to both particle classification and health significance. NIOSH	

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Peer Reviewer 4 (cont.)		is conducting field studies that include measurement of CNT/CNF "structure" counts (classified in different categories) and EC mass (and other metrics) and will assess the merits of the different metrics as additional toxicological data become available. However, a method based on tubes/cc or total CNT/CNF 'structures' ignores the many complex agglomerates typical of CNT/CNF aerosols.	