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Peer Review of the NIOSH Draft CIB: Occupational Exposure to Carbon Nanotubes and Nanofibers

NIOSH should be commended for providing a much-needed analysis of all the data available on carbon nanotube and carbon nanofiber hazards to derive occupational exposure limits for these materials.

reviewed the CIB and offered comments that are included along with the comments of Please consider the following:

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?** 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. 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.

B. 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:

1. **Is the use of Co levels in lungs for CNT lung burden estimations, per Pauluhn (2010a) preferable to the current CIB approach?** The CIB estimates the retained lung burden in rats from the Pauluhn study using the MPPD 2.0 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.

2. **Is there a need to cite cardiovascular effects literature related to these nanomaterials in greater detail?** 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 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).

3. **Additional information on CNFs could be supplied to support CNF inclusion in the NIOSH CIB.** 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.

4. **Is the benchmark dose modeling approach appropriate for the derivation of an OEL, as opposed to using NOAELs from the two subchronic inhalation studies?** 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).

   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 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.

5. **Appropriate considerations for the POD:** 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 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.)

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, 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).

In this CIB, in fact, the data themselves do not appear to support any modeling. Consider two instances illustrating this point in the CIB:

> 1) 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.)

2) 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.

6. **The CIB should review Pauluhn (2010b) which derived a different OEL for MWCNTs based on the same data used in the CIB.** 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 data – 0.05 mg Baytubes/m^3 as a time weighted average -- a discussion of these different approaches, and why the CIB value is more appropriate, should be offered.

7. **Was the dichotomization of fibrotic effects done appropriately?** 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.

   While the discussion immediately above provides a biological argument against the choice of cutpoint selected by NIOSH, there is also a statistical argument against grouping the response severities as done by NIOSH. The CIB infers that 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 cutpoint of grade 2 should be used.

8. **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.** 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.

   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 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.

C. 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.

Thank you for the opportunity to comment.

References:

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USEPA. 2000. Benchmark Dose Technical Guidance Document (External Review Draft). EPA/630/R-00-0001 October 2000.

USEPA. 2009. Toxicological Review of Cerium Oxide and Cerium Compounds. EPA/635/R-08/002F, please see *www.epa.gov/iris.*

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[i] This paper does not necessarily reflect the views and policies of the
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