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September 5, 2022

Ms. Heather Tenney
TURI Program Manager
The Offices at Boott Mills West
126 John Street, Suite 14
Lowell, MA 01852

SENT BY: e-mail to Heather_Tenney@uml.edu

RE: Petition to add single-walled and multi-walled carbon nanotubes (CNTs), and carbon nanofibers (CNFs) to the Toxic Use Reduction Act Toxic or Hazardous Substance List - TURA Science Advisory Board Call for Information

Dear Ms. Tenney:

As a manufacturer of single-walled carbon nanotubes (SWCNTs) in Massachusetts, Nano-C respectfully submitted information on May 13, 2022 and on June 15, 2022 in response to the above referenced "call for information." On May 26, 2022 and on June 29, 2022, the Science Advisory Board (SAB) met to review and discuss scientific research conducted on SWCNTs. As a member of the public, Nano-C attended both meetings. A third meeting of the SAB has been scheduled for September 16, 2022 to further examine the pulmonary toxicology data on SWCNTs and to determine if a recommendation should be made to the Administrative Council to list SWCNTs to the Massachusetts Toxic Use Reduction Act's (TURA) Toxic or Hazardous Substance List.

We respectfully provide these additional comments to supplement our previous letters of May 13, 2022 and June 15, 2022, and to support information discussed during the June 29th SAB meeting, specifically the concept of particle lung overload and its relevance to the SWCNT scientific research literature being reviewed and considered by the SAB. We thank you again for the opportunity to provide comments, and to demonstrate that SWCNTs do not qualify for listing.

Executive Summary

Excessively high inhalation and bolus doses and dose rates used in the majority of pulmonary rodent toxicity studies of SWCNTs overwhelm the animals' normal clearance processes resulting in adverse health effects associated with particle lung overload. In addition, existing pulmonary toxicity studies have not been executed in accordance with recommended guidelines. Existing data are inadequate for use as the basis for a listing recommendation.

Nonetheless, if the TURI SAB determines a listing recommendation is necessary, the proposed listing should be "*Single Walled Carbon Nanotubes, airborne, unbound particles of respirable size*" which properly describes the substance evaluated.

Particle Lung Overload Phenomenon

Discussed in our June 15, 2022 letter, the extremely high inhalation and bolus doses and dose rates used in pulmonary rodent toxicity studies of SWCNTs likely overwhelmed the animals' normal clearance processes resulting in "lung overload."

> "*Particle overload (also referred to as `lung overload` or `clearance overload`) describes a condition of slowed/impaired (macrophage mediated) clearance in the lung after prolonged exposure to poorly soluble particles of low inherent toxicity. This condition is further characterised by an increased transfer of particles to lymph nodes, accumulation of particles in the lung, increases in lung weight, pulmonary inflammation, epithelial hyperplasia (proliferation), fibrosis and eventually cancer (in the rat)*[1]*. A rat-specific effect pattern can be assumed as evidenced by greater pulmonary inflammatory -, fibrotic -, hyperplastic – and particularly a unique tumorigenic responses to particle exposures as compared to other species (e.g. mice, hamster, non-human primates, humans).*"[2]
>
> "*First introduced by Morrow in 1988, [i]t is now well established that lung effects following chronic inhalation to PSPs [*poorly soluble particles*] of low toxicity occur only at exposures which are concurrently leading to an accumulation of particles in the deep lung as a result of significant impairment of pulmonary particle clearance."*[3]
>
> *"This cascade of events runs primarily at exposures to high particle concentrations and thus* ***may be considered a result of the experimental set-up rather than a true reflection of the virtually low intrinsic toxic potential of PSP*** [Emphasis added]. *The term "high particle concentration" has not been clearly defined but is related to the amount of poorly soluble material deposited daily in the lungs, and thus, the pulmonary clearance rate seems to be a useful indicator to approximate the critical exposure concentration(s) resulting in lung overload conditions. Analysing results from various lung clearance tests in rats and hamsters exposed for several months to a variety of particulate aerosols led to the conclusion that lung clearance is retarded by chronic exposure to respirable particles at concentrations of 3 mg/m*^{3} *or higher (Muhle et al, 1988). A similar concept of a so called "critical deposition rate" was based on mathematical analyses of lung clearance rates by Yu et al, 1989 and was defined as "rate above which the overload condition will be present if the exposure time is sufficient". An alternative definition of*

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[1] Specifically, adenomas and carcinomas in the peripheral lung of rats.
ECETOC, *Poorly Soluble Particles/Lung Overload*, Technical Report No. 122, Brussels, 2013, pp. 3.
ISSN-0773-8072-122 (Print); ISSN-2073-1526-122 (Online)

[2] Ibid., pp. 7.

[3] ECETOC, 2013, pp. 4.

"critical deposition rate" may be seen in the threshold dose leading to impaired alveolar macrophage mediated lung clearance, which is equivalent to approximately 1 mg per gram lung tissue[4,5] (Morrow, 1988) or 1 µl per gram of lung (Oberdörster, 1995).[6]

Scientific and regulatory experts agree, "[*l*]*ung particle overload has been demonstrated in all laboratory animal species tested."*[7]

Examination of the Shvedova mice studies:

Applying the findings of Muhle et al., the exposure dose of 5 mg/m^3 administered to the experimental animals in the Shvedova studies[8,9] **exceeds** the lung clearance concentration range. In the Shvedova studies, *"[t]he chain of pathological events … was realized through synergized interactions of early inflammatory response and oxidative stress culminating in the development of multifocal granulomatous pneumonia and interstitial fibrosis."*[10] These adverse effects experienced by the laboratory animals (mice) in the Shvedova studies (2008 & 2013) likely are due to particle overload conditions resulting from the excessive dose of SWCNTs administered in these studies.

Examination of the Morimoto rat study:

Applying the findings of Muhle et al., the exposure doses of 0.03 mg/m^3 and 0.13 mg/m^3 administered to the experimental animals (rats) in the Morimoto study **are below the concentration** at which lung clearance is retarded. In this "*4-week inhalation study of well-dispersed purified SWCNTs, [n]either [the] low nor high concentrations of SWCNTs induced inflammation of mainly neutrophils or the concentration of CINCs [cytokine-induced neutrophil chemoattractant] or HO-1 [*heme oxygenase-1] *in the lung. Well-*

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[4] Weight range of the lung of laboratory mice is 0.171 grams – 0.215 grams (depending on animal's species and gender). Diehl, L and Morse, M.*, A Comparison of Selected Organ Weights and Clinical Pathology Parameters in Male and Female CD-1 and CByB6F1 Hybrid Mice 12-14 Weeks in Age,* Charles River, no year. https://www.criver.com/sites/default/files/resources/AComparisonofSelectedOrganWeightsandClinicalPathologyParametersinMaleandFemaleCD-1andCByB6F1HybridMice12-14WeeksinAge.pdf

[5] Weight range of the lung of laboratory rats is 1.48±0.29 - 2.43±0.49 grams (depending on animal's age and gender). Piao, Y., Liu, Y., and Xie, X., *Change Trends of Organ Weight Background Data in Sprague Dawley Rats at Different Ages*, J. Toxicol Pathol., Vol. 26, 2013, pp. 29–34.

[6] ECETOC, 2013, pp. 3-4.

[7] Driscoll, KE. and Borm, PJA., *Expert workshop on the hazards and risks of poorly soluble low toxicity particles,* Inhalation Toxicology, Vol. 32, No. 2, 2020, pp. 53-62.

[8] Shvedova, AA., et al., *Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis*, Am J Physiol Lung Cell Mol Physiol., Vol. 295, 2008, pp. 552-565. doi:10.1152/ajplung.90287.2008

[9] Shvedova, AA., et al., *Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: one-year postexposure comparisons*, Am J Physiol Lung Cell Mol Physiol., Vol. 306, 2013, pp. 170–182. doi:10.1152/ajplung.00167.2013

[10] Shvedova, AA., et al., 2008

dispersed SWCNTs did not induce neutrophil inflammation in the lung under the conditions in the present study."[11] The laboratory animals in the Morimoto study were **not** exposed to particle overload conditions.

Caution must be used in the interpretation of bolus exposure study results

Experimental design of both inhalation and instillation studies may result in lung overload conditions experienced by the laboratory rat, mice and hamsters if dose rates are excessive, that is, the study's dose exceeds the guidance provided by Morrow[12], Muhle et al.[13], or Oberdorster[14] noted above.

Recognizing it is not always feasible to conduct the preferred inhalation study, in 2000, the Inhalation Specialty Section of the Society of Toxicology elected to develop a document to summarize key issues concerning the use of instillation as an exposure technique for the evaluation of respiratory tract toxicity in rodents. The authors of this paper, titled *"Forum; Intratracheal Instillation as an Exposure Technique for the Evaluation of Respiratory Tract Toxicity: Uses and Limitations,"* find …

> *"Perhaps the most consistently reported disparity between inhalation and intratracheal instillation methods relates to the intrapulmonary distribution of particles. Inhalation results in a relatively homogeneous distribution of particles throughout the lungs, whereas instillation generally results in less homogeneity of dose distribution in the alveolar region and can result in focally high doses of material. These differences in dose distribution can influence clearance pathways, doses to certain cells and to tissues, and the degree and site of systemic absorption*.
> …
> "*A thorough understanding of the differences between the instillation and inhalation methods is necessary to avoid misinterpretation of instillation-derived results. A key difference between the two exposure methods is the dose rate, i.e., administration of a dose within a few seconds with intratracheal instillation, as opposed to minutes, hours, days, weeks, or even months when the material is inhaled. The possibility of delivering excessive doses to the lungs because of the bolus*

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[11] Morimoto, et. al, *Pulmonary toxicity of well-dispersed single-wall carbon nanotubes after inhalation,* Nanotoxicology, Vol. 6(7), November 2012, pp. 766–775.
doi: 10.3109/17435390.2011.620719

[12] Morrow, PE., *Possible mechanisms to explain dust overloading of the lungs,* Fundamental and Applied Toxicology Vol. 10, 1988, pp. 369–841.

[13] Muhle, H., Bellmann, B., Heinrich U., *Overloading of lung clearance during chronic exposure of experimental animals to particles*, Annals Occupational Hygiene, Vol. 32, 1988, pp. 141-147.

[14] Oberdorster, G., *Lung Particle Overload: Implications for Occupational Exposures to Particles*, Regulatory Toxicology and Pharmacology, Vol. 27, 1995, pp. 123-135.

delivery inherent in an instillation exposure poses the risk of overwhelming lung defenses and causing effects that are not relevant to those which may occur at lower doses or dose rates.[15]

Oberdorster et al.,[16] concur, stating …

> "*A main difference is the delivery of material either as a bolus exposure (instillation, aspiration) or by inhalation, with the former representing a nonphysiological mode of delivery of the liquid suspended material within a fraction of a second (very high dose rate), whereas the latter physiological inhalation deposits aerosolized materials over an extended period of time (days, weeks, or months, termed low dose rate).*
> …
> *A high dose rate and high doses may overwhelm normal defense mechanisms and thus result in significant initial pulmonary inflammation, and may also affect disposition of the administered material to secondary organs*."[17]

The large majority of the pulmonary toxicology data for SWCNTs have been generated via intratracheal instillation & aspiration studies in rodents. Oberdorster et al., have determined the bolus exposures used in these SWCNT studies have "*result[ed] in high lung burdens at **a very high dose rate** [Emphasis added].*"[18,19] "*Responses induced by such high doses are likely due to mechanisms*, *such as particle overload or effects of homeostasis, that are not operative at relevant low doses.*"[20]

Given their limitations, caution **must be exercised** when considering the results of SWCNT bolus exposure studies for hazard classification.

Existing Data are Inadequate as the Basis for a Listing Recommendation

As stated in our June 15, 2022 comments, in light of the design of the majority of pulmonary bolus exposure toxicology studies, Oberdorster, et al., recommend an appropriately designed 13-wk inhalation

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[15] Driscoll, KE., Costa, DL., Hatch, G., Henderson, R., Oberdorster, G., Salem, H., and Schlesinger, RB., *Forum; Intratracheal Instillation as an Exposure Technique for the Evaluation of Respiratory Tract Toxicity: Uses and Limitations,* Toxicological Sciences, Vol. 55, 2000, pp. 24 –35.

[16] Oberdorster, G., Castranova, V., Asgharian B., & Sayre, P., *Inhalation Exposure to Carbon Nanotubes (CNT) and Carbon Nanofibers (CNF): Methodology and Dosimetry*, J. Toxicol Environ Health – Part B Crit Review, Vol. 18(0), 2015, pp. 121–212. doi:10.1080/10937404.2015.1051611.

[17] Ibid, pp. 123.

[18] Oberdorster, G., et al., 2015, pp. 128.

[19] See Nano-C's June 15, 2022 comments to TURI's Scientific Advisory Board Table 1, which provides a "Comparison of Animal Inhalation Toxicology Study Exposures to Years of Worker Exposure."

[20] Oberdorster, G., et al., 2015, pp. 123.

studies in rodents[21] be conducted to evaluate the effects of SWCNTs and suggest *"[l]ung burdens from reported bolus exposure studies may be used as guidance for determination of aerosol exposure concentration (ideally resulting in low, medium, and high doses)*."[22] In addition to this specific data need for SWCNTs, based on their critical review of the research, the authors of Oberdorster et al. (2015) provide general recommendations applicable to CNTs and CNFs *that address test guideline modifications for rodent inhalation studies that will improve dosimetric extrapolation modeling for hazard and risk characterization based on the analysis of exposure-dose-response relationships.*[23] A summary[24] of these guidelines is as follows:

- Inhalation is the preferred method to obtain data necessary for risk assessment of pulmonary exposure to CNT/CNF
- The rat is the preferred rodent species
- Whole-body exposure is less stressful and thus preferred over nose-only exposure, in particular for 90-d and longer inhalation studies
- At present, 90-day inhalation studies are, with CNT/CNF aerosol characterization according to Organization for Economic Cooperation and Development/US Environmental Protection Agency general guidelines for performing a sub-chronic study
- Use a minimum of three concentrations that include a NOAEL (i.e., no-observed-adverse-effect level) and an MTD (i.e., maximum tolerated dose). Determination of retained lung burden is essential in order to express results based on dose-response data rather than only as exposure-response relationship
- Inclusion of a postexposure observation/recovery period is highly desirable
- Precede the 90-day inhalation study with an acute 1- to 10-day inhalation study with 3 or more concentrations in order to identify effects; estimate exposure concentrations for sub-chronic 90-day study; and verify aerosol characteristics and evenness of chamber distribution and general tolerance of the animals to exposure
- A 28-day inhalation exposure may provide sufficient information with added post-exposure observation/recovery time

Although published in 2012, the pulmonary toxicology study of well-dispersed SWCNTs after inhalation conducted by Morimoto, et al., was not evaluated by Oberdorster, et al. in their 2015 review. As noted above, the exposure doses of 0.03 mg/m^3 and 0.13 mg/m^3 administered to the experimental animals (rats) in the Morimoto study **are below the concentration** at which lung clearance is retarded, resulting in no induction of *neutrophil inflammation in the lung.* However, the Morimoto study does not meet all the recommendations outlined by Oberdorster et al. (2015) for an "appropriately designed 13-wk inhalation studies in rodents" to assess SWCNT toxicity, nor does it meet many of the general test guidelines for the evaluation of CNTs by pulmonary studies noted above.

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[21] Inhalation toxicology studies are considered to be the gold standard when assessing pulmonary response to an agent.

[22] Oberdorster, G., et al., 2015, pp. 128.

[23] Ibid., pp. 121.

[24] Oberdorster, G., et al., 2015, pp. 179-180.

Given these deficiencies, as stated in our June 15, 2022 comments, caution must be exercised in using the existing laboratory animal results as the basis of a recommendation to list SWCNTs to the Massachusetts TURA Toxic or Hazardous Substance List. Without results from appropriately designed **inhalation** study(ies), a recommendation to list SWCNTs by the SAB would be premature. A subsequent decision by the Administrative Council to add SWCNTs to the TURA Toxic or Hazardous Substance List would place undue and unnecessary burden on manufacturers and users of SWCNTs within the Commonwealth of Massachusetts to comply with listing requirements. In addition, a premature, erroneous listing of SWCNTs would adversely and irreparably stigmatize this substance as a toxic/hazardous material.

A Recommendation to List must Consider the Form of the Substance

Nonetheless, if the TURI SAB determines a listing recommendation has merit, the proposed listing must be limited to the specific form of the substance evaluated in animal pulmonary studies - that is, *"single walled carbon nanotubes: airborne, unbound particles of respirable size."* This qualifying language properly describes the substance that has been evaluated.

The genesis of this qualifying language is the California Office of Environmental Health Hazard Assessment's (OEHHA) listing of two poorly soluble particles, carbon black and titanium dioxide, under its Safe Drinking Water and Toxic Enforcement Act of 1986, also known as Proposition 65. The OEHHA recognizes the exposure circumstance evaluated and associated with the laboratory animal response. When these particles are bound within a matrix, not able to become airborne, they do not present a pulmonary hazard.

Conclusion

In closing, based on the current existing scientific data, SWCNTs **do not qualify** for listing to the Toxic Use Reduction Act Toxic or Hazardous Substance List.

We would be pleased to respond to any questions or provide additional information to the TURI SAB. We thank you again for the opportunity to provide comments.

Respectfully,

Viktor Vejins
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Nano-C

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Jerome Lang
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