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Nano-C, Inc.
33 Southwest Park
Westwood, MA 02090
Tel 781.407.9417
Fax 781.407.9419
www.nano-c.com

June 15, 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 in response to the above referenced "call for information." On May 26, 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 the May 26, 2022 meeting. A second meeting of the SAB has been scheduled to further examine the pulmonary toxicology data on SWCNTs and to determine if a recommendation will 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 clarify information stated during the May 26th SAB meeting, and to provide important and relevant information related to the 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

The SWCNT dose levels used in the majority of pulmonary toxicity studies conducted on laboratory animals exceed, by orders of magnitude, dose levels that are expected under realistic inhalation exposure in the workplace. The adverse responses seen in the animals from these excessively high doses are likely due to particle overload or the effects of homeostasis. Thus, these 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.

Update of Substance Characteristics

During the May 26, 2022 SAB meeting Mr. Tom Lada, Vice President of Operations at Nano-C, assisted the SAB in understanding the magnitude of exposure levels used in the laboratory animal research studies by noting SWCNTs are low density materials. As stated by the ISO Technical Committee on Nanotechnologies, *"[t]he density … of CNTs and CNFs is important because this property is one of the main factors that influence the aerodynamic behaviour and deposition fractions in the lungs. The aerodynamic properties for deposition of CNT and CNF aerosols larger than 0,3 µm*[1] *are especially influenced by the density, whereas particles less than 100 nm are not influenced by the density. Because the density is highly correlated with the volume, the volume per unit mass increases as the density decreases. This can induce volumetric overload in cells, especially in phagocytic cells."*[2]

Mr. Lada informed the SAB that the density of Nano-C's "as produced material is typically 0.03 kg/L;" this is a low-density material. The typical characteristics of Nano-C's purified SWCNT product line have been updated to include density and water solubility:

Length	< 0.5 micron	(Scanning Electron Microscopy)
Diameter	0.9 to 1.3 nm	(Raman Spectroscopy)
Carbon	95%	(Thermogravimetric Analysis)
Iron/Iron Oxide	≤ 5%	(Thermogravimetric Analysis)
SWCNT	> 99% (of carbon)	(Absorbance by Ultra-violet Visible Spectroscopy)
Amorphous Carbon	< 1%	(Absorbance by Ultra-violet Visible Spectroscopy)
Density (as produced)	0.03kg/L	(Tap density; ASTM B527-22)
Water Solubility	Insoluble	

Laboratory Animal Exposures to SWCNTs are Excessive and Do Not Represent Human Exposures

"*Increasing awareness of the importance of physicochemical properties as determinants of toxicity of CNT/CNF and existing difficulties in interpreting results of mostly acute rodent inhalation studies*" have

[1] "0,3 µm" is a direct quote from the ISO Technical Report cited in footnote 2 below. As the document has been published in Switzerland, the authors have used a comma instead of a decimal point. For USA readers, the number is 0.3 µm.

[2] ISO/TR 23463Technical Report, *Nanotechnologies — Characterization of carbon nanotube and carbon nanofibre aerosols to be used in inhalation toxicity tests*, International Organization for Standardization, Switzerland, 2022, pp 10.

necessitated a critical review of CNF and CNT research and a reexamination of standardized inhalation testing guidelines by the authors of Oberdorster et al. 2015.[3] Of importance, the authors note,

> *"[w]hen assessing potential effects of airborne CNT and CNF in animal studies,* ***equivalent human exposure conditions ideally need to be mimicked*** *[Emphasis added] by considering exposure methods and mode and dosimetric aspects*." …
>
> *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. …* ***The impact of high doses also needs to be considered when the amounts exceed by orders of magnitude dose levels that are expected to be deposited in the respiratory tract of humans under realistic inhalation exposure scenarios*** *[Emphasis added]. The selection of high bolus doses is often justified by arguments that the delivered dose is the same—per unit alveolar surface area—as is deposited per unit alveolar surface area in humans exposed to occupational exposure levels over a 40-yr working life. This ignores completely the effect of dose rate, that is, delivery of the same dose over a long period (days, months, years) versus within a fraction of a second …. 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 ….*[4]

To examine the critical parameter of dose, a comparative analysis has been performed. Laboratory animal (e.g., mice and rats) exposure dosages used in selected pulmonary toxicology studies have been compared to the US National Institute for Occupational Safety and Health's (NIOSH) Recommended Exposure Limit (REL) for CNT and CNF of 1 $\mu g/m^3$ (8-hr. TWA).[5] The results of this analysis, presented in Table 1, demonstrate animal exposures **far exceed** the Recommended Exposure Limit (REL) for CNT/CNF. Most pulmonary studies of SWCNTs involved bolus exposure, resulting in high lung burdens at a very high dose rate.[6]

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[3] Oberdorster, G., et al., *Inhalation Exposure to Carbon Nanotubes (CNT) and Carbon Nanofibers (CNF): Methodology and Dosimetry*, J Toxicol Environ Health B Crit Rev. 2015; 18(0): 121–212. doi:10.1080/10937404.2015.1051611.

[4] Oberdorster, G., et al., 2015.

[5] Department of Health & Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, *Current Intelligence Bulletin 65, Occupational Exposure to Carbon Nanotubes and Nanofibers*, April 2013.

[6] Oberdorster, G., et. al., 2015.

Table 1
Comparison of Animal Inhalation Toxicology Study Exposures to Years of Worker Exposure

Study / Type of Study	Animal Exposure	Comparison to the CNT/CNF NIOSH REL for Workers*
[7,8]Shvedova, et al., 2008 & 2013 / Inhalation (mouse)	5 mg/m^3 (5hr/day for 4 days)	730 years of worker exposure
[9]Shvedova, et al., 2005 / Pharyngeal aspiration (mouse)	10 µg/mouse 20 µg/mouse 40 µg/mouse (single exposure)	31 years for worker exposure (10 µg) 63 years for worker exposure (20 µg) 126 years for worker exposure (40 µg)
[10]Mangum et al., 2006 / Pharyngeal aspiration (rat)	2 mg/kg (single exposure)	167 years of worker exposure
[11]Kobayashi et al., 2011 / Intratracheal instillation (rat)**	0.2 mg/kg 1.0 mg/kg 2.0 mg/kg (single exposure)	17 yrs for worker exposure (0.2 mg/kg) 83 yrs for worker exposure (1.0 mg/kg) 167 yrs for worker exposure (2.0 mg/kg)

*Assumes 8-hr exposure/day for 250 working days per year
**Kobayashi, et al., exposures are representative of other intratracheal instillation studies
Appendix A provides details on the comparative analysis

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[7] Shvedova, A.A., 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 295, 2008, pp. 552-565. doi:10.1152/ajplung.90287.2008

[8] Shvedova, A.A., 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 306, on-line 2013, pp. 170–182. doi:10.1152/ajplung.00167.2013

[9] Shvedova, A.A., et al., *Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice*, Am. J. Physiol. Lung Cell. Mol. Physiol. 2005., 289, pp 698-708.

[10] Mangum, J.B., et al., 2006*. Single-walled carbon nanotube (SWCNT)-induced interstitial fibrosis in the lungs of rats is associated with increased levels of PDGF mRNA and the formation of unique intercellular carbon structures that bridge alveolar macrophages In situ.*, 2006, Part. Fibre Toxicol. 3 (15). http://dx.doi.org/10.1186/1743-8977- 3-15.

[11] Kobayashi, N., et al., *Pulmonary and systemic responses of highly pure and well-dispersed carbon nanotubes after intratracheal instillation*. Inhal. Toxicol. 2011, Vol. 23, pp. 814-828. DOI: 10.3109/08958378.2011.614968

Morimoto, et al., provides a contrasting inhalation study[12] with laboratory rats exposed at lower dose rates which found no increases of total cell or neutrophil counts in the bronchoalveolar lavage fluid, or the concentration of cytokine-induced neutrophil chemoattractant in the lungs or bronchoalveolar lavage fluid ninety-days after exposure.[13] Also of note are the findings for the lowest exposure of 0.04 mg/kg in the intratracheal instillation study conducted in rats by Kobayahi, et al., - no significant difference in lung weight compared to the control group; no abnormality in necropsy findings, no observed changes in BALF inflammatory cells; and, no significant changes observed in BALF biomarkers. A comparative analysis, presented in Table 2, determines animal exposures within the Morimoto and Kobayashi Studies more closely align with human exposures.

Table 2
Comparison of Animal Inhalation Toxicology Study Exposures to Years of Worker Exposure

Study / Type of Study	Animal Exposure	Comparison to the CNT/CNF NIOSH REL for Workers*
Morimoto, et al. / Inhalation (rat)	0.03 mg/m^3 (6 hr/day for 5 days for 4 wks)	4 years of worker exposure
	0.13 mg/m^3 (6 hr/day for 5 days for 4 wks)	16 years for worker exposure
Kobayashi et al., 2011 / Intratracheal instillation (rat)	0.04 mg/kg (single exposure)	3 yrs for worker exposure

*Assumes 8-hr exposure/day for 250 working days per year

Under conditions of heavy exposure, normal clearance processes become overwhelmed, and the total lung burden of particles accumulates at a rate faster than that predicted under normal conditions; this phenomenon has been referred to as lung overload. Rats and other rodent species exhibit lung overload, but it has not been observed in humans. *For example, overload doses of poorly soluble particles (PSP) of low toxicity overwhelm the alveolar macrophage clearance function, which [has been] shown to induce lung tumors in rats.*[14]

We conclude that the extremely high bolus dose and dose rates of the majority of the SWCNT pulmonary toxicology studies conducted in rodents do not compare to dose-response relationships of realistic inhalation exposures, nor have equivalent human exposure conditions been mimicked in these

[12] Inhalation studies are considered the gold standard in evaluating pulmonary toxicity.

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

[14] Oberdorster, et al., 2015.

studies. SWCNTs are poorly soluble particles of low density; the extremely high bolus dose and dose rates used in these rodent studies likely overwhelmed the animals' normal clearance processes. As such, these conditions are not representative of human exposures that are reasonably expected in the workplace.

Existing Data are Inadequate as the Basis for a Listing Recommendation

In light of the design of the majority of pulmonary toxicology studies, Oberdorster, et al., recommend an appropriately designed 13-wk inhalation studies in rodents[15] 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)*."[16]

In the absence of 13-week study data, caution must be exercised in using the existing laboratory animal data as the basis of a recommendation to list SWCNTs to the Massachusetts TURA Toxic or Hazardous Substance List. Without results from appropriately designed studies - that is, studies at relevant doses that eliminate the mechanisms of particle overload or effects of homeostasis – 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 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 recognized 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.

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[15] Inhalation toxicology studies are considered to be gold standards when assessing pulmonary response.

[16] Oberdorster, et al., 2015.

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 these comments.

Respectfully,

Viktor Vejins
President & CEO
Nano-C

Thomas Lada
Vice President of Operations
Nano-C

Jerome Lang
Safety, Health & Environmental
Manager, Nano-C

Appendix A

Analysis of Animal Research Exposure to Human Exposure

Example below uses mouse exposure protocol in Shvedova, et al., (2008 & 2013) studies

Dose in Mouse Study	5000	µg/m^3	
Dose in Mouse Study	0.005	µg/mL	
Average Mouse Mass*	26.5	g	
Average Mouse Respiration Rate**	1.46	mL / g mouse min	
Study Exposure	5	hours/day	
Study Exposure	4	days	
Mouse respiration	38.69	mL/min	
Total exposure Time	1200	minutes	
Total Exposure Volume	46428	mL	
Total Absolute Dose	232.14	µg	
Dose per body weight	8760	µg/kg	
NIOSH CNT/CNF REL	1	µg/m^3	
Average Human Breath	0.5	L	
Human Weight	75	kg	
Average Human Respiration Rate	15	breaths/min	
Target Dose from Study above	8760	µg/kg	
Absolute Dose for human	657000	µg	Target dose from study divided by human weight
Amount of Air @ REL	657000	m^3	
			Amount of air @ REL divided by volume of human breath
# of Breaths Required	1314000000	breaths	
Required Exposure Time	87600000	minutes	
	1460000	hours	
	182500	8-hr Working Days	
			8-hr working days divided by OSHA standard of 250 working days per year
	730	**yrs. of exposure**	

* https://web.jhu.edu/animalcare/procedures/mouse.html

** https://www.ncbi.nlm.nih.gov/pmc/articles/PMC380668/