May 13, 2022

Materials That Power Our World

Ms. Heather Tenney
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Lowell, MA 01852

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

RE: Comments in response to TURI's call for information on carbon nanotubes and carbon nanofibers

Dear Ms. Tenney:

We understand the Toxic Use Reduction Institute's (TURI's) Administrative Council has received a 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. The petitioners have requested the reporting threshold be reduced to 100 grams, and the CNTs and CNFs be listed as higher hazard substances.[1] Further, we understand the TURA Science Advisory Board (SAB) has begun the process of reviewing these substances and is seeking additional information from stakeholders.[2] As producers of single-walled carbon nanotubes (SWCNTs), we respectfully submit the following information on SWCNTs, and thank you for the opportunity to demonstrate that SWCNTs do not qualify for listing.

Executive Summary

The GreenScreen® Hazard Assessment commissioned by the TURA SAB identified five health human and environmental endpoints of potential concern for adverse effects associated with exposure to SWCNTs. Review of reliable scientific data for each of these endpoints has resulted in the following determinations with the conclusion that SWCNTs do not qualify for listing under the Massachusetts Toxic Use Reduction Act's Toxic or Hazardous Substance List.

[1] *Petition to Toxics Use Reduction Act Administrative Council*, from Clean Water Action & Public Employees for Environmental Responsibility, to Toxic Use Reduction Institute's (TURI's) Administrative Council, June 24, 2020. https://www.turi.org/content/download/13331/204352/file/Petition_to_Toxics_Use_Reduction_Act_Administrative_Council__4_.pdf

[2] TURI website announcement, May 26, 2021. https://www.turi.org/Our_Work/Policy/Toxics_Use_Reduction_Act/Councils_and_Committees/TURA_Science_Advisory_Board/Call_for_Information_on_Carbon_Nanotubes_and_Carbon_Nanofibers

Endpoint	Nano-C's Determination
Mutagenicity/Genotoxicity	The weight of evidence does not support the mutagenicity/genotoxicity of SWCNTs
Systemic Toxicity/Organ Effects incl. Immunotoxicity (repeat dose for lung)	Insufficient evidence exists for adverse pulmonary effects of SWCNTs. SWCNTs do not meet the "extended fiber toxicology paradigm"
Eye Irritation/Corrosivity	Scientific evidence does not support SWCNTs as irritating or corrosive to the eye
Chronic Aquatic Toxicity (ecotoxicity)	Conflicting evidence for adverse aquatic toxicity of SWCNTs. More data are needed
Persistence (environmental fate)	Insufficient evidence exists for environmental persistence of SWCNTs

Introduction

Nano-C is a leading innovator in the chemistry of nanostructured carbon, including single-walled nanotubes. Headquartered in Westwood, Massachusetts, Nano-C was founded in 2001. Its materials are critical elements in emerging high-value applications in the electronics and energy markets by supporting the constant demand for devices that are energy efficient, smaller, lighter, more versatile and durable. Nano-C's SWCNTs are manufactured in a proprietary selective and continuous combustion process initially developed at the Massachusetts Institute of Technology (MIT). In 2015, Nano-C received conditional clearance by the U.S. Environmental Protection Agency (EPA) to commercially manufacture specific SWCNT products. It is important to note that EPA's conditional clearance binds Nano-C's customers to the same conditions applicable to Nano-C via a mandatory agreement that must be in place as a condition for domestic commercial sales. To date, production of Nano-C's SWCNTs remains for research and development purposes only, however commercial sales are expected. To our knowledge, Nano-C is one of a limited number manufacturers of SWCNTs within Massachusetts.

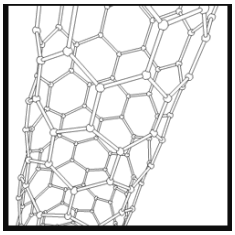
The Substance

The International Organization for Standardization (ISO) defines a nanotube as a "hollow fibre."[3] ISO defines a single-walled carbon nanotube as a, "carbon nanotube consisting of a single cylindrical

[3] ISO/TS 27687:2008, *Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate*, International Organization for Standardization, 2008, 4.4.

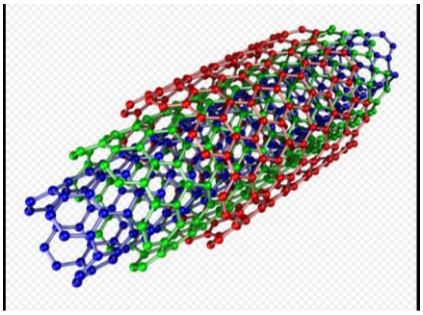
ISO defines nanofiber as a, "*nano-object* (3.1.3) with two external dimensions in the nanoscale (3.1.1) and the third dimension significantly larger.

graphene layer" noting "[t]he structure can be visualized as a graphene sheet rolled into a cylindrical honeycomb structure.[4] The graphene sheet is one-atom thick, wrapped into a seamless cylinder with either open or closed ends.



Source: SWCNT image. https://upload.wikimedia.org/wikipedia/commons/7/76/Kohlenstoffnanoroehre_Animation.gif

The single cylindrical graphene layer differentiates SWCNTs from multi-walled carbon nanotubes (MWCNTs). ISO defines a MWCNT as a "carbon nanotube composed of nested, concentric or near-concentric graphene layers with interlayer distances similar to those of graphite."[5]



Source: MWCNT image. https://en.wikipedia.org/wiki/Carbon_nanotube#/media/File:Multi-walled_Carbon_Nanotube.png

Due to this structural difference, the physical properties of SWCNTs and MWCNTs differ significantly from each other in three primary ways. First, SWCNTs are not rigid materials at the molecular level, discerning them from the rigidity of MWCNTs and other known respirable hazardous materials like asbestos. Second, as discussed in more detail below, the length of SWCNTs is typically 5 microns or less, less than MWCNT which are often in the 10-30 micron range, and significantly less than that of nanofibers known to be hazardous, as discussed in detail below. And third, due to van de Waals interactions and entanglements, manufactured SWCNTs are highly agglomerated, bundles of flexible

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Note 1 to entry: The largest external dimension is not necessarily in the nanoscale.
Note 2 to entry: The terms "nanofibril" and "nanofilament" can also be used.
Note 3 to entry: See 3.1.3, Note 1 to entry. Nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger"

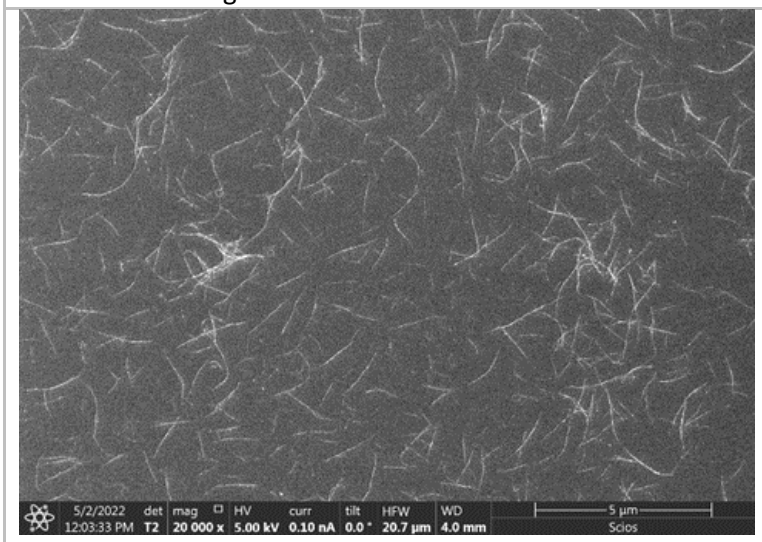
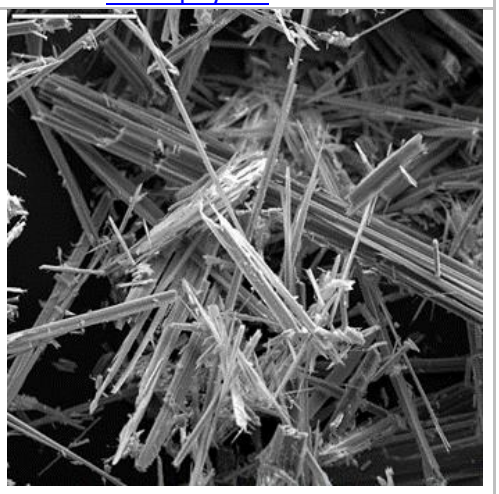
ISO/TS 80004-3:2020, *Nanotechnologies - Vocabulary - Part 3: Carbon nano-objects*, International Organization for Standardization, 2020, 3.1.7.

[4] ISO/TS 80004-3:2020 Nanotechnologies - Vocabulary - Part 3: Carbon nano-objects, International Organization for Standardization, 2020, 3.3.4.

[5] Ibid., 3.3.6.

tubes and typically are purified (to remove the metal catalyst required to manufacture the carbon nanotube), exfoliated and dispersed in a liquid prior to use.

These intrinsic properties influence the toxicology profile of SWCNTs as discussed below.

Single-Walled Carbon Nanotubes	Anthophyllite Asbestos
	
Source: Scanning electron microscope image of highly dispersed SWCNTs produced by Nano-C as imaged by the Kostas Advanced Nano-Characterization Facility of Northeastern University, Burlington, MA SWCNT Sample Preparation: SWCNTs were dissolved, then centrifuged; the supernatant was diluted with a drop of supernatant then deposited on a silicon chip, dried then imaged.	Source: Scanning electron microscope image from [http://usgsprobe.cr.usgs.gov/picts2.html]

SWCNTs have unique properties that make them an essential additive in a variety of formulations to advance performance because SWCNTs:

- May be either semi-conducting or metallic
- Are stronger than steel, yet lighter than aluminum
- Conduct heat most efficiently
- Can easily be modified to tailor properties as an "Ink"

Due to their superior electrical conductivity, strength, temperature and chemical stability, SWCNTs form the basis for key electronic applications such as memory, semiconductor components and transparent conducting films for touch screens, displays, solar cells, sensors, elastomers, thermosets and thermoplastics.[6,7] To achieve the desired performance in these applications, SWCNTs may be used at

[6] Nano-C's website.
https://nano-c.com/technology-platform/what-is-a-nanotube/

[7] OCSiAl's Tuball™ product website.
https://tuball.com/articles/single-walled-carbon-nanotubes

loading rates much, much lower than conventional additives (e.g., > 0.01% by weight SWCNTs compared to 0.5- 5% by weight MWCNTs compared to 15-35% for metal fillers).[8]

The main methods of nanotube synthesis include.[9]

- plasma arc discharge: production of carbon nanotubes in an arc discharge plasma in a helium atmosphere
- laser evaporation (pulsed laser ablation): evaporation of a graphite target in a high-temperature reaction by the action of a pulsed laser
- chemical vapor deposition (CVD): catalytic (nickel, cobalt, iron or their combinations) deposition of carbon from hydrocarbons (methane, ethylene, acetylene)
- combustion of hydrocarbons (methane, ethanol, acetylene, for example) in the presence of a catalyst (iron, nickel, cobalt or combinations)

SWCNTs are used exclusively as an additive; no application uses CNTs on their own. SWCNTs are placed on the market in powder form, a wet-cake or paste for ease of handling, or as a highly purified ink. These SWCNT products are then incorporated/encapsulated into customer-specific formulations for use in devices; unencapsulated materials do not reach the consumer.

Strict U.S. Regulations Govern Commercial Manufacture and Use of SWCNTs

Under the U. S. Toxic Substance Control Act (TSCA), if a substance is listed on the chemical inventory, said substance would not be considered a "new" chemical, thus it would not be subject to the requirement to submit a premanufacture notice (PMN) to the U.S. Environmental Protection Agency (EPA) prior to commercial manufacture/sale or import. In October 2008, the EPA deviated from this standard by determining it "generally considers CNTs to be chemical substances distinct from graphite or other allotropes of carbon listed on the TSCA Inventory. Many CNTs may therefore be new chemicals under TSCA section 5. Manufacturers or importers of CNTs not on the TSCA Inventory must submit a premanufacture notice (PMN) (or applicable exemption) under TSCA section 5 where required …."[10]

Morphology, composition, and purity may vary amongst CNTs. By requiring the submission of PMNs for all "new" CNTs, the Agency has the opportunity to assess the hazard profiles of each commercially manufactured and imported CNTs and issue a consent order should it be determined that the substance presents an unreasonable risk of injury to human health and/or the environment.

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[8] Ibid.

[9] OCSiAl's Tuball™ product website.

[10] Federal Register, Vol. 73, No. 212, October 31, 2008, pp. 64946 - 64947. https://www.federalregister.gov/documents/2008/10/31/E8-26026/toxic-substances-control-act-inventory-status-of-carbon-nanotubes

Since the October 2008 Notice, CNTs have been the subject of strict regulations by the EPA. To our knowledge,[11] the Agency has issued a consent order for each CNT that is the subject of a submitted PMN. Standard consent order terms of which we have direct knowledge include all or most of the conditions noted below:

- Use limitations for very specific applications, only
- Prohibition on application methods that generate dust, mist or aerosol unless said method occurs in an enclosed process
- Mandatory use of defined NIOSH-approved inhalation and dermal personal protective equipment
- Mandatory generation of physical-chemical, toxicology and/or ecotoxicology data, usually including a 90-day inhalation study in laboratory rats
- Prohibited release to water
- Mandatory recordkeeping requirements
- Distribution restriction of CNTs only to downstream users who agree to abide by the same above-mentioned conditions, expect mandatory testing

Typically, the requirement to generate the specified test data is tied to the commercial manufacture of a specific volume of the PMN CNT, or to a specific time period post commencement of commercial manufacture.

Once the CNT has been reviewed and cleared by the EPA with a signed consent order in place, the Agency has the authority to issue a Significant New Use Rule (SNUR) which requires notice to the EPA before the CNT is used in a new manner that might create concerns.[12] The Agency has issued Significant New Use Rules (SNURs) for both single-walled carbon nanotubes and multi-walled carbon nanotubes.[13, 14]

Notably, the EPA has exempted from some SWCNT and MWCNT SNURs, the "quantities of the substance [CNTs] that have been embedded or incorporate into a polymer matrix that itself has been reacted (cured); embedded in a permanent solid polymer form that is not intended to undergo further processing, except mechanical processing; or incorporated into an article…".[15] This

[11] The specific decisions and actions by EPA on PMNs are confidential.

[12] U.S. Environmental Protection Agency Website, *Actions Under TSCA Section 5*. https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/actions-under-tsca-section-5#SNURs

[13] Federal Register, Vol. 84, December 5, 2019, pp. 66598. https://www.govinfo.gov/content/pkg/FR-2019-12-05/pdf/2019-26225.pdf

[14] Federal Register, Vol. 76, May 6, 2011, pp. 26186 - 26192. https://www.federalregister.gov/documents/2011/05/06/2011-11127/multi-walled-carbon-nanotubes-significant-new-use-rule

[15] Ibid.

exemption recognizes these matrices “bind” the CNTs thus minimizing or eliminating exposure and release potential.

Manufacturing Control & Consistent Purity & Morphology Profile

As noted above, SWCNTs are high value, specialty chemical additives that are engineered to improve existing performance properties. The development of a commercial SWCNT product, that meets application-specific performance criteria, is a thoughtful, reiterative process that involves deep knowledge of the application and close cooperation with a potential customer. During the development process, critical feedback from the customer is used to determine the required purity profile of the SWCNT and its morphology. Once a research and development grade of SWCNT achieves established performance targets under laboratory conditions, the potential customer will evaluate the material in a deliberate, detailed qualification process to confirm the SWCNT will achieve all performance targets in its commercial formulation(s). Should the formulated SWCNT meet all targets, the SWCNT is “locked,” that is, manufacturing specifications are frozen to ensure the purity and morphology profile of the SWCNT do not change. Development of a commercial SWCNT product takes many months to years of effort. This effort often results in an exclusive customer-specific product as the refined SWCNTs are unlikely to be suitable in other customer applications.

Achieving performance criteria for the key applications[16] served by Nano-C's SWCNTs typically requires very low residual iron concentration. Typical purity characteristics of a purified SWCNT product line are:

Length	< 0.5 micron	(Scanning Electron Microscopy)
Diameter	0.9 to 1.3 nm	(Raman Spectroscopy)
Carbon	95%	(Thermogravimetric Analysis)
Iron/Iron Oxide	$\leq$ 5%	(Thermogravimetric Analysis)
SWCNT	> 99% (of carbon)	(Absorbance by Ultra-violet Visible Spectroscopy)
Amorphous Carbon	< 1%	(Absorbance by Ultra-violet Visible Spectroscopy)

Scientific review

As will be presented, scientific data do not support the listing of SWCNTs to the Toxic Use Reduction Act Toxic or Hazardous Substance List; definitive evidence is lacking.

In June of 2021, ToxServices LLC completed a hazard assessment, called a GreenScreen®, at the request of the Massachusetts Toxic Use Reduction Institute’s Science Advisory Board. The GreenScreen® assessment of SWCNTs identified the following human health and environmental endpoints of possible concern:

[16] Carbon electronics, Sensors, Displays & Touch Screens

- Mutagenicity/Genotoxicity
- Systemic Toxicity/Organ Effects incl. Immunotoxicity (Repeat dose for lung)
- Eye Irritation/Corrosivity
- Chronic Aquatic Toxicity (ecotoxicity)
- Persistence (environmental fate)

These endpoints are the focus of the discussion below.

Mutagenicity/Genotoxicity

Determination: Based on our review of the data, we believe the weight of evidence does not support the mutagenicity/genotoxicity of SWCNTs.

Review of in vitro and in vivo studies:

- In vitro gene mutation in bacteria studies, "reliable without restriction" [Klimisch (KL) Score 1], showed no evidence of cytotoxicity/increase in mutation frequency.[17]
- In vitro cytogenicity or micronucleus formation studies demonstrated mixed results. Reliable studies (KL 1 & 2) showed no evidence of increased chromosomal aberrations with or without metabolic activation, and no increase in reactive oxygen species (ROS) production. Other studies "reliable with restrictions" (KL 2) showed DNA damaging effects produced via the generation of ROS.[18]
- Results from in vitro gene mutation in mammalian cells research are not considered, as application of the KL score criteria determined these studies to be of the lowest reliability, that is KL Score of 4.[19]
- In vivo mutagenicity studies demonstrated mixed results; however, the quality/reliability of the data must be examined. In several reliable studies (KL Score 1 & 2), SWCNTs:
  - Were not genotoxic in the comet assay following intratracheal instillation in rats
  - Did not induce increases in micronucleus frequency at any dose
  - Did not evoke significant in vivo micronuclei frequencies in mice polychromatic erythrocytes
  - Did not show any potential for genotoxic activity in the in vivo micronucleus assay
  - Showed no significant changes in the percent tail deoxyribonucleic acid were found in any group exposed to long and short SWCNT[20]

[17] Muller, J., *Literature search report related to single-wall carbon nanotubes (SWCNT)*, ToxMinds B.V.B.A., March 11, 2022, pp. 21-23.

[18] Ibid., pp. 23-32.

[19] Muller, J., pp. 32-33.

[20] Ibid., pp. 33-36.

In one "reliable study with restrictions" (KL 2), and several other studies classified as "not reliable" (KL3), SWCNTs:

- Induced elevated level of DNA damage measured as % DNA in the tail
- Increased levels of 8-oxo-2'deoxyguanosine in lung and liver
- Increase in mitochondrial DNA damage
- Increased mutation frequency in mice[21]

Per the GreenScreen® Hazard Assessment, "SWCNTs were assigned a score of Moderate for mutagenicity/genotoxicity based on positive results for DNA damage seen in in vivo assays conducted with [two types of] SWCNT[s] … leading the WHO[22] work group to classify the entire SWCNT category to GHS Category 2.[23] GreenScreen® criteria classify chemicals as a Moderate hazard for mutagenicity/genotoxicity when they are classified to GHS Category 2…."[24] The GreenScreen® Hazard Assessment concluded, "**[t]he confidence in the score is low due to the limited evidence and lack of Guideline DNA damage studies on various grades of SWCNTs** [Emphasis added]."[25]

In conclusion, the weight of evidence does not support the mutagenicity/genotoxicity of SWCNTs.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (Repeat dose for lung)

Determination: Based on our review of the data, we believe insufficient evidence exists for adverse pulmonary effects of SWCNTs.

As discussed below, SWCNTs do not meet the "extended fiber toxicology paradigm" - SWCNTs are not long, rigid or biopersistent. As will be discussed in the Environmental Fate Section, enzymatic peroxidase assisted mechanisms biodegrade SWCNTs.

Subacute toxicology studies by Matsumoto et al. showed no adverse effects.[26] One OECD Guideline study noted increased neutrophil cells in blood at 3 months after administration in the high

[21] Ibid., pp. 36-38.

[22] WHO - World Health Organization

[23] United Nations' Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Category 2 germ cell mutagens are those that cause concern owing to the possibility that they may induce heritable mutations in germ cells of humans.

[24] *Single Walled Carbon Nanotubes (SWCNTs) (CAS #308068-56-6) GREENSCREEN® For Safer Chemicals (GREENSCREEN®) Assessment,* ToxServices LLC, June 10, 2021, pp. 9.

[25] Ibid.

[26] Morimoto Y, et al., 2012. *Pulmonary toxicity of well-dispersed single-wall carbon nanotubes after inhalation*, Nanotoxicology, Vol. 6, Issue 7, 2012. pp. 766-775.

concentration group (i.e., 0.40 mg/m^3). However, a second OECD Guideline study found no adverse pulmonary effects or signs of neutrophil inflammation in the high dose group (i.e., 0.13 mg/m^3).[27]

One type of SWCNTs, Tuball™, could not be aerosolized to conduct a subchronic inhalation study.[28] The two intratracheal instillation studies conducted by Kobayashi et al., "showed inflammatory responses related with the vital reaction to the foreign substance that was instilled intratracheally, and there were no fibrosis, atypical lesion, or tumor-related findings even at the highest dose (2 mg/kg) of SWCNT-exposed groups up to 6 months after instillation. The inflammatory response was not induced in other tissues (i.e., the liver, kidney, spleen, and cerebrum)."[29]

Per the GreenScreen® Hazard Assessment, "SWCNTs were assigned a score of High for systemic toxicity (repeated dose) based on WHO's classification of the entire SWCNT group to GHS Category 1[30] following repeated inhalation exposure with the lung as the target organ. GreenScreen® criteria classify chemicals as a High hazard for systemic toxicity (repeated dose) when they are classified to GHS Category 1 ..."[31] The GreenScreen® Hazard Assessment concluded, "**[t]he confidence in the score is low as the available evidence for classification were considered weak by the WHO** [Emphasis added]"[32]

Further, examination of the "extended fiber toxicology paradigm" sheds light on the inhalation toxicology study findings for SWCNTs.

> "The fiber toxicology paradigm addresses fiber inhalation-related hazards. It was first proposed by Pott and Friedrichs and Stanton and Wrench on the basis of toxicological findings on asbestos, mineral and vitreous fibers and can be formulated as

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[27] *Single Walled Carbon Nanotubes (SWCNTs) (CAS #308068-56-6) GREENSCREEN® For Safer Chemicals (GREENSCREEN®) Assessment*, pp. 15.

[28] EU REACH registration dossier for single-walled carbon nanotubes (EC number: 943-098-9), Toxicology information.
NOTE: No stable atmosphere in air could be achieved by various methods. The six different technical measures for dust generation were tested: fluid bed generator; brush generation without cyclone; brush generator with cyclone; brush generator with sieving unit and cyclone; brush generator with jet-mill and cyclone; and, Wright dust generator. All failed to generate a respirable and stable dust atmosphere of the test material, allowing the conduct of inhalation testing in a reliable manner.
https://echa.europa.eu/registration-dossier/-/registered-dossier/18023/7/6/3

[29] Kobayashi N, et. al., *Pulmonary and systemic responses of highly pure and well-dispersed single-wall carbon nanotubes after intratracheal instillation in rats,* Inhalation Toxicology, Vol. 23, 2011, pp. 814-828.

[30] GHS Category 1 - Reliable evidence on the substance of an adverse effect on specific organ/systems or systemic toxicity in humans or animals

[31] *Single Walled Carbon Nanotubes (SWCNTs) (CAS #308068-56-6) GREENSCREEN® For Safer Chemicals (GREENSCREEN®) Assessment*, pp. 15.

[32] Ibid.

> 'The elongated shape of fibers is a carcinogenic principle, provided the fibers are respirable, long and biodurable.'

According to this paradigm, nanomaterials that are fiber-shaped (aspect ratio > 3), respirable (diameter < 3 µm), long (length > 5 µm) and biopersistent may pose harm to lungs. …

In a series of studies, Donaldson et al. observed that macrophages failed to ingest CNTs longer than 15 µm, causing injuries of cell membranes (lesions). This initiated inflammatory cell response, similar to effects known from long asbestos fibers. On the other hand, neither short nor tangled CNTs were observed to induce similar effects, which supports the hypothesis that short (<5 µm), flexible CNTs or tangled agglomerates of CNTs are less harmful. Nagai et al. likewise studied the role of fiber [rigidity] in contact with lung cells and found that tangled, non-rigid multi-walled carbon nanotubes (MWCNTs) caused less harm, whereas non-agglomerated rigid nanotubes, both thin and thick, caused stronger damage, both in macrophages and the mesothelium. The important role of high bending modulus and rigidity has recently been highlighted also by Kane et al. 2018. High flexural rigidity is believed to be associated with cell lesions and incomplete phagocytosis.

For future interpretation of toxicological data on fibers, [Fortini et al.] propose to substitute the parameter "fiber diameter" by "fiber rigidity" … extending the fiber toxicology paradigm, which intrinsically is a material-independent hypothesis, by the following hypothesis that also includes flexural rigidity:

> 'The elongated shape of fibers is a carcinogenic principle, provided the fibers are respirable, long, rigid and biodurable.' [33]

SWCNTs do not meet the criteria of the extended fiber toxicology paradigm. SWCNTs are flexible, agglomerated bundle of nanotubes, and at least one type of SWCNT, Tuball™ cannot be aerosolized.

In conclusion, insufficient evidence exists for adverse pulmonary effects of SWCNT.

Eye Irritation/Corrosivity

Determination: Based on our review of the data, we believe scientific evidence does not support SWCNTs as irritating or corrosive to the eye.

Two reliable studies with restrictions (KL 2) (2011 Ema et al. and 2020 Kim et al.), found SWCNTs were "not founded to be corrosive, irritant or a skin sensitizer."[34]

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[33] Renata Fortini, R., et al*., Measurement of Flexural Rigidity of Multi-Walled Carbon Nanotubes by Dynamic Scanning Electron Microscopy,* Fibers, Vol. 8, Issue 31, May 12, 2020. https://www.baua.de/EN/Service/Publications/Essays/article2808.pdf?__blob=publicationFile&v=1

[34] Muller, J., *Literature search report related to single-wall carbon nanotubes (SWCNT)*, ToxMinds B.V.B.A., March 11, 2022, pp. 8.

Per the GreenScreen® Hazard Assessment, "SWCNTs were assigned a score of High for eye irritation/corrosivity based on irritating effects seen in an in vitro ocular irritation study conducted with Tuball™ SWCNT classifying it to GHS Category 2A[35]. GreenScreen® criteria classify chemicals as a High hazard for eye irritation/corrosivity when they are classified to GHS Category 2A …."[36]

The GreenScreen® assessment concludes, "**[t]he confidence in the score is low due to the limitation in the in vitro OECD Guideline 492 study as it is only recommended for identifying substances not requiring classification for eye irritation or serious eye damage** [Emphasis added]. The test cannot discriminate eye irritants (Category 2) from substances causing serious eye damage (Category 1)…."[37]

In conclusion, scientific evidence does not support SWCNTs as irritating or corrosive to the eye.

Chronic Aquatic Toxicity

Determination: Based on our review of the data, we believe evidence for adverse aquatic toxicity of SWCNTs is conflicting. More data are needed.

Acute aquatic toxicity (for completeness, acute data are discussed)
Two acute OECD Guideline studies of Daphnia magna and Pseudokirchneriella subcapitata exposed to Tuball™ SWCNT found no toxic effects at saturation.[38] However, a well-documented, non-guideline freshwater study of Escherichia coli observed cell damaging effects after 4 days of exposure to Tuball™ SWCNTs as recorded by bioluminescence measurements over a period of 14 days.[39]

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[35] A substance is classified as Eye Irritant Category 2A (irritating to eyes) when it produces in at least in 2 of 3 tested animals a positive response of:
(i) corneal opacity ≥1; and/or
(ii) iritis ≥1; and/or
(iii) conjunctival redness ≥2; and/or
(iv) conjunctival edema (chemosis) ≥2
calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the substance, and which fully reverses within an observation period of normally 21 days.

[36] *Single Walled Carbon Nanotubes (SWCNTs) (CAS #308068-56-6) GREENSCREEN® For Safer Chemicals (GREENSCREEN®) Assessment*, pp. 19.

[37] *Ibid*.

[38] EU REACH registration dossier for single-walled carbon nanotubes (EC number: 943-098-9), Ecotoxicology information.

[39] Ibid.

Chronic aquatic toxicity

"SWCNT were assigned a score of High for chronic aquatic toxicity based on measured LOEC [Lowest Observed Effect Concentration] values of 1 mg/L in daphnia and algae for two types of SWCNT … GreenScreen® criteria classify chemicals as a High hazard for chronic aquatic toxicity when chronic aquatic toxicity values are > 0.1 to 1.0 mg/L ...."[40] The GreenScreen® assessment concludes, "**[t]he confidence in the score is low due to lack of study details of the observed effects** [Emphasis added]."[41]

In conclusion, there is conflicting evidence for adverse aquatic toxicity of SWCNTs. More data are needed.

Environmental Fate, Persistence

Determination: Based on our review of the data, we believe insufficient evidence exists for environmental persistence of SWCNTs.

In-vitro and in vivo scientific studies [42,43,44,45] describe "that there are different enzymatic peroxidase assisted mechanisms that biodegrade single wall carbon nanotubes, finally leading to carbon dioxide by such oxidative clearance mechanism. It appears that BSA (blood serum proteins) and different oxidase enzymes such as lactoperoxidase, horseradish peroxidase, lignin peroxidase and myeloperoxidase all were confirmed degrading single wall carbon nanotubes in vivo. Such mechanisms do take place … also in the environment, and thus it can be assumed that corresponding clearance mechanisms in the environment do also take place, finally degrading such single wall carbon nanotubes to carbon dioxide."[46]

[40] *Single Walled Carbon Nanotubes (SWCNTs) (CAS #308068-56-6) GREENSCREEN® For Safer Chemicals (GREENSCREEN®) Assessment,* pp. 21.

[41] Ibid.

[42] Kotchey, G. P., et al., *A natural vanishing act: the enzyme-catalyzed degradation of carbon nanomaterials,* Acc. Chem. Res. Vol. 45, 2021, pp. 1770–1781. doi: 10.1021/ar300106h

[43] Kotchey, G. P., et al., *Peroxidase-mediated biodegradation of carbon nanotubes in vitro and in vivo,* Adv. Drug Deliv. Rev., Vol. 65, 2013, pp. 1921–1932. doi: 10.1016/j.addr.2013.07.007

[44] Allen, B.L., et al., *Biodegradation of Single-Walled Carbon Nanotubes through Enzymatic Catalysis*, Nano Letters, Vol. 8, 2008, pp. 3899–3903. doi: 10.1021/nl802315h

[45] Allen, B. L., et al. *Mechanistic investigations of horseradish peroxidase-catalyzed degradation of single-walled carbon nanotubes*, J. Am. Chem. Soc., Vol. 131, 2009, pp. 17194–17205. doi: 10.1021/ja9083623

[46] EU REACH registration dossier for single-walled carbon nanotubes (EC number: 943-098-9), PBT assessment.

Per the GreenScreen® Hazard Assessment, "SWCNTs were assigned a score of Very High for persistence based on no biodegradation seen in various biodegradation studies conducted according to OECD Guidelines and GLP [Good Laboratory Practices] with two types of SWCNT …. In addition, SWCNTs are non-volatile inorganic materials, and therefore not expected to partition to the air. In water, soil and sediment, they are expected to be recalcitrant without undergoing biotic or abiotic degradation. GreenScreen® criteria classify chemicals as a Very High hazard for persistence when they are recalcitrant in the environment."[47] The GreenScreen® assessment concludes, "**[t]he confidence in the score is reduced as no experimental data are available that last more than 28 days** [Emphasis added]."[48]

SWCNTs are an insoluble, inorganic material. Classical biodegradability and bioaccumulation guideline studies are not applicable to inorganic material such as SWCNTs. In addition, enzymatic peroxidase assisted mechanisms biodegrade SWCNTs.

In conclusion, insufficient evidence exists for environmental persistence of SWCNTs.

Conclusion

In closing, we trust the information provided has been helpful. Based on the foregoing review of 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,

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President & CEO
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Safety, Health & Environmental
Manager, Nano-C

[47] *Single Walled Carbon Nanotubes (SWCNTs) (CAS #308068-56-6) GREENSCREEN® For Safer Chemicals (GREENSCREEN®) Assessment*, pp. 21.

[48] Ibid.