nano-c®
nanostructured carbon
Materials That Power Our World

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

November 22, 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:

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]

As a manufacturer of single-walled carbon nanotubes (SWCNTs) in Massachusetts, Nano-C respectfully has submitted information on May 13, 2022, June 15, 2022 and September 5, 2022 in response to the above referenced "call for information." On May 26, 2022, June 29, 2022 and September 16, 2022, the Science Advisory Board (SAB) met to review and discuss scientific research conducted on SWCNTs. As a member of the public, Nano-C has attended all three meetings. A fourth meeting of the SAB has been scheduled for December 8, 2022 to continue further examination of 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.

[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

We respectfully provide these summary comments to emphasize the critical information presented in our past three submissions. Nano-C believes this key scientific information and expert opinions demonstrate **SWCNTs do not qualify for listing**.

Summary of critical scientific data and expert opinions

SWCNTs are not inherently hazardous; definitive scientific evidence is lacking for a hazard determination.

- SWCNTs do not meet the extended fiber toxicology paradigm which states:
  - *The elongated shape of fibers is a carcinogenic principle, provided the fibers are respirable, long, rigid and biodurable*.[2]
  - SWCNTs are flexible, agglomerated bundles of nanotubes. SWCNTs are not biodurable; i*n-vitro* and *in vivo*, enzymatic peroxidase assisted mechanisms biodegrade SWCNTs.[3,4,5,6]

- The adverse responses induced by excessively high dose rate and high doses administered in the vast majority of instillation and aspiration studies are due likely to mechanisms such as **particle overload[7] or the effects of homeostasis**, that are not operative at relevant low doses.[8]
  - The delivered dose to the animal, within a fraction of a second, far **exceeds the per unit alveolar surface area in humans exposed to occupational exposure levels over a 40-yr working life** (see

[2] 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

[3] 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

[4] 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

[5] 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

[6] 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

[7] See Appendix A for details of the *Particle Lung Overload Phenomenon*

[8] 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.

Appendix B). These bolus dose administration methods ignore completely the effect of dose rate[9]

- *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*[10]
- Pulmonary toxicology experts agree intratracheal instillation studies *can be problematic as it results in the focal deposition of material*[11,12]
- *When 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*[13]
- Further, "*[t]he density … of CNTs and CNFs … is one of the main factors that influence the aerodynamic behaviour and deposition fractions in the lungs. ... 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*** [Emphasis added]."[14] SWCNTs are a low-density material; the density of Nano-C's as produced SWCNTs is typically 0.03 kg/L

T**he adverse pulmonary effects found in the high dose instillation and aspiration studies '*may be considered a result of the experimental set-up rather than a true reflection of the virtually low intrinsic toxic potential of PSP [poorly soluble particles].*[15]** SWCNTs are poorly soluble particles.

---

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

[10] 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. 123. doi:10.1080/10937404.2015.1051611.

[11] 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.
https://pubmed.ncbi.nlm.nih.gov/32149535/

[12] 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.

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

[14] "*The aerodynamic properties for deposition of CNT and CNF aerosols larger than 0,3 µm are especially influenced by the density."* The typical length of Nano-C's purified SWCNTs is < 0.5 micron.
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.

[15] ECETOC, *Poorly Soluble Particles/Lung Overload*, Technical Report No. 122, Brussels, 2013, pp. 3.
ISSN-0773-8072-122 (Print); ISSN-2073-1526-122 (Online)

- Relevant low doses SWCNT inhalation and intratracheal instillation studies found no adverse effects in laboratory animals
  - [L]*ung clearance is retarded by chronic exposure to respirable particles at concentrations of 3 mg/m^3 or higher (Muhle et al, 1988) … the threshold dose leading to impaired alveolar macrophage mediated lung clearance, which is equivalent to approximately 1 mg per gram lung tissue*[16,17] *(Morrow, 1988) or 1 µl per gram of lung (Oberdörster, 1995)*[18]
  - **The Morimoto et al. rat inhalation study 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**[19] The exposure doses of 0.03 mg/m^3 and 0.13 mg/m^3 administered to the experimental animals **are below the concentration** at which lung clearance is retarded
  - Kobayashi et al. (2011) the lowest exposure of 0.04 mg/kg in the intratracheal instillation study conducted in rats found 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.[20] This single exposure doses administered to the experimental animals **is below the concentration** at which lung clearance is retarded.
  - Further, the doses administered to the laboratory animals in the Morimoto and Kobayshi studies are relevant to worker exposures – see Appendix B

- As per the guidelines outlined by expert pulmonary toxicologists Oberdorster et al., a well-designed 13-wk inhalation toxicology study in rats has not been conducted/published
  - Only two inhalation studies have been identified in the peer reviewed literature – Shvedova (2008) and Morimoto
  - Shvedova (2008) study administers an exposure dose of 5 mg/m^3 to the laboratory mice which **exceeds** the lung clearance concentration range

---

[16] 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

[17] 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.

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

[19] 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

[20] 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 study administers two exposure doses to the laboratory rats; both doses are **below** the concentration at which lung clearance is retarded

An inhalation study fulfilling the recommendations of Oberdorster, et al. is needed to obtain data necessary for risk assessment of pulmonary exposure to SWCNTs[21]

A Recommendation to List is Unfounded and Premature

Inappropriate study design has led to the findings of adverse health effects in laboratory animals exposed to SWCNTs due to the well documented particle lung overload phenomenon. More appropriately designed studies find no adverse effects in laboratory animals exposed to SWCNTs.

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 an appropriately designed **inhalation** study(ies), a recommendation to list SWCNTs by the SAB would be unfounded and 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."* Note, all three listing qualifiers - airborne, unbound (not bound within a matrix), and respirable size (10 micrometers or less in diameter) - must be met for SWCNTs to be considered a listed substance under the Massachusetts Toxic Use Reduction Act's (TURA) Toxic or Hazardous Substance List.

Precedence exists for the listing of substances with qualifying language. In 2000, crystalline silica was added to the Massachusetts Toxic Use Reduction Act's (TURA) Toxic or Hazardous Substance List with the qualification, *particle sizes less than 10 microns*.[22]

The proposed qualifying language for SWCNTs is pertinent as these poorly soluble particles do not present a pulmonary hazard when bound within a matrix, and not able to become airborne.

---

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

[22] *TURA List of Toxic or Hazardous Substances Background Document for discussion by the TURA Ad Hoc Committee, April 29, 2021*
https://www.mass.gov/doc/tura-ad-hoc-committee-background-document-tura-toxic-or-hazardous-substances-list/download

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 thank you again for the opportunity to provide 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
Particle Lung Overload Phenomenon

"*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)*[23]*. 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).*"[24]

"*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.*"[25]

"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 "critical deposition rate" may be seen in the threshold dose leading to impaired*

[23] 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)

[24] Ibid., pp. 7.

[25] ECETOC, 2013, pp. 4.

alveolar macrophage mediated lung clearance, which is equivalent to approximately 1 mg per gram lung tissue[26,27] *(Morrow, 1988) or 1 µl per gram of lung (Oberdörster, 1995).*[28]

Scientific and regulatory experts agree, "[*l*]*ung particle overload has been demonstrated in all laboratory animal species tested.*"[29]

[26] 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

[27] 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.

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

[29] 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.

Appendix B
***Summary of SWCNT Pulmonary Tox*icology Studies**

NOTE: OSHA standard of 250 working days per year
1 µg/m^3 is current NIOSH Recommended Exposure Limit (REL)

Author/ Year	Title	Dose (Animal)	Key Effects[30]	Equivalent Human Dose[31]
INTRATRACHEAL INSTILLATION STUDIES				
Lam/ 2004	*Pulmonary Toxicity of SWCNTs in Mice 7 and 90 Days After Intratracheal Instillation*	0, 0.1, 0.5 mg (Mouse)	All nanotube products induced dose-dependent epithelioid granulomas and, in some cases, interstitial inflammation	At 0.1 mg, equivalent to 79,000 eight-hour work days at 1 µg/m^3 equaling **314 yrs of exposure**. At 0.5 mg, equivalent to 390,000 eight-hour work days at 1 µg/m^3 equaling **1,572 yrs of exposure**.
Park / 2011	*A single intratracheal instillation of single-walled carbon nanotubes induced early lung fibrosis and subchronic tissue damage in mice*	100 µg/kg (Mouse)	*… a single intratracheal instillation of SWCNTs may induce early lung fibrosis and subchronic tissue damage.* (Quote from Abstract)	At 100 µg/kg, equivalent to 2,100 eight-hour work days at 1 µg/m^3 equaling **8 yrs of exposure**.
Chang / 2011	*Epithelial-mesenchymal transition contributes to SWCNT-induced pulmonary fibrosis*	80 µg (Mouse)	Pulmonary epithelial and mesenchymal injury, followed by granulomatous and fibrotic changes	At 80 µg, equivalent to 63,000 eight-hour work days at 1 µg/m^3 equaling **252 yrs of exposure**.
Park / 2016	*Single-walled carbon nanotubes disturbed the immune and metabolic regulation function 13-weeks after a single intratracheal instillation*	50, 100, and 200 µg/kg (Mouse)	Disturbed immune and metabolic regulation functions	At 50 µg/kg, equivalent to 1,000 eight-hour work days at 1 µg/m^3 equaling **4 yrs of exposure**. At 100 µg/kg, equivalent to 2,100 eight-hour work days at 1 µg/m^3 equaling **8 yrs of exposure**.

[30] *Key Effects* as summarized by TURI Program Manager H. Tenney unless otherwise noted
[31] As calculated by Nano-C

				At 200 µg/kg, equivalent to 4,200 eight-hour work days at 1 µg/m^3 equaling **17 yrs of exposure**.
Kobayashi / 2011	*Pulmonary and systemic responses of highly pure and well-dispersed SWCNTs after intratracheal instillation in rats*	.04, 0.2, 1, 2 mg/kg (Rat)	*The histopathological findings in the lungs of rats exposed to SWCNTs 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 (2mg/kg) of SWCNT exposed groups up to 6 months after instillation.* (Quote from Abstract)	At 0.04 mg/kg, equivalent to 830 eight-hour work days at 1 µg/m^3 equaling **3 yrs of exposure**. At 0.2 mg/kg, equivalent to 4,200 eight-hour work days at 1 µg/m^3 equaling **17 yrs of exposure**. At 1 mg/kg, equivalent to 21,000 eight-hour work days at 1 µg/m^3 equaling **83 yrs of exposure**. At 2 mg/kg, equivalent to 42,000 eight-hour work days at 1 µg/m^3 equaling **167 yrs of exposure**.
Fujita / 2015	*Size effects of single-walled carbon nanotubes on in vivo and in vitro pulmonary toxicity*	0.18 mg/kg, 1.8 mg/kg (Rat)	Numerous genes were significantly upregulated or downregulated	At 0.18 mg/kg, equivalent to 3,750 eight-hour work days at 1 µg/m^3 equaling **15 yrs of exposure**. At 1.8 mg/kg, equivalent to 38,000 eight-hour work days at 1 µg/m^3 equaling **150 yrs of exposure**.
Ema / 2017	*Length effects of single-walled carbon nanotubes on pulmonary toxicity after intratracheal instillation in rats*	1 mg/kg (Rat)	BALF, lung injury, inflammation	At 1 mg/kg, equivalent to 21,000 eight-hour work days at 1 µg/m^3 equaling **83 yrs of exposure**.

Honda / 2017	*A 104-week pulmonary toxicity assessment of long and short single-wall carbon nanotubes after a single intratracheal instillation in rats*	0.2, 1 mg/kg for long SWCNTs 1 mg/kg for short SWCNTs (Rat)	*Inflammatory changes, test substance deposition, test substance engulfment by macrophages, and alveolar wall fibrosis were observed in the lungs of almost all test rats at 52 and 104 weeks after short nanotube instillation. The incidences of these changes were much lower in the long nanotube-treated groups. In almost all rats of the long nanotube-treated groups, fibrosis and epithelium loss in the terminal bronchiole with test substance deposition were observed. These bronchiolar changes were not observed after administering short nanotubes.* (Quote from Abstract)	At 0.2 mg/kg, equivalent to 4,200 eight-hour work days at 1 µg/m^3 equaling **17 yrs of exposure**. At 1 mg/kg, equivalent to 21,000 eight-hour work days at 1 µg/m^3 equaling **83 yrs of exposure**.
Zhang / 2011	*Functionalized single-walled carbon nanotubes cause reversible acute lung injury and induce fibrosis in mice*	0.06, 0.2, 0.6, 2, and 10 mg/kg (Mouse)	some f-SWCNTs could induce acute lung injury (ALI) in mice via proinflammatory cytokine storm signaling through the NF-κB pathway in vivo. ... corticosteroid treatments could ameliorate the ALI induced by the f-SWCNTs in mice. … the ALI was almost completely reversed within 14 days, while mild to moderate fibrosis, granuloma, and DNA damage remained in the mice at day 14.	At 60 µg/kg, equivalent to 1,300 eight-hour work days at 1 µg/m^3 equaling **5 yrs of exposure**. At 200 µg/kg, equivalent to 4,200 eight-hour work days at 1 µg/m^3 equaling **17 yrs of exposure**. At 600 µg/kg, equivalent to 13,000 eight-hour work days at 1 µg/m^3 equaling **50 yrs of exposure**. At 2,000 µg/kg, equivalent to 42,000 eight-hour work days at 1 µg/m^3 equaling **167 yrs of exposure**. At 10,000 µg/kg, equivalent to 210,000 eight-hour work days at 1 µg/m^3 equaling **833 yrs of exposure**.

INTRAPHARYNGEAL INSTILLATION				
Li / 2007	*Cardiovascular Effects of Pulmonary Exposure to Single-Wall Carbon Nanotubes*	10, 40 µg single administra-tion 20 µg repeated doses for 8 wks (Mouse)	*A single intrapharyngeal instillation ... induced activation of heme oxygenase-1 (HO-1) in lung, aorta, and heart tissue. … mice exposed to SWCNT (10 and 40 µg/mouse), developed aortic mtDNA damage at 7, 28, and 60 days after exposure. mtDNA damage was accompanied by changes in aortic mitochondrial glutathione and protein carbonyl levels. Because these modifications have been related to cardiovascular diseases, we evaluated whether repeated exposure to SWCNTs (20 µg/mouse once every other week for 8 weeks) stimulates the progression of atherosclerosis in ApoE–/– transgenic mice. Although SWCNT exposure did not modify the lipid profiles of these mice, it resulted in accelerated plaque formation in ApoE–/– mice fed an atherogenic diet. Plaque areas in the aortas, measured by the en face method, and in the brachiocephalic arteries, measured histopathologically, were significantly increased in the SWCNT-treated mice. This response was accompanied by increased mtDNA damage but not inflammation* (Quote from Abstract)	At 10 µg, equivalent to 7,900 eight-hour work days at 1 µg/m^3 equaling **31 yrs of exposure**. At 40 µg, equivalent to 31,000 eight-hour work days at 1 µg/m^3 equaling **126 yrs of exposure**. … At 20 µg / m^3, equivalent to 16,000 eight-hour work days at 1 µg/m^3 equaling **63 yrs of exposure**.
PHARYNGEAL ASPIRATION				
Teeguarden / 2011	*Comparative Proteomics and Pulmonary Toxicity of Instilled Single-Walled Carbon Nanotubes, Crocidolite Asbestos, and Ultrafine Carbon Black in Mice*	40 µg twice a week for 3 weeks (Mouse)	Histologically, the incidence and severity of inflammatory and fibrotic responses were greatest in mice treated with SWCNTs	At 40 µg, equivalent to 31,000 eight-hour work days at 1 µg/m^3 equaling **126 yrs of exposure**.

Shvedova / 2005	*Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice*	0, 10, 20, 40 µg (Mouse)	A rapid progressive fibrosis found in mice exhibited two distinct morphologies: 1) SWCNT-induced granulomas mainly associated with hypertrophied epithelial cells surrounding SWCNT aggregates and 2) diffuse interstitial fibrosis and alveolar wall thickening likely associated with dispersed SWCNT. *dose dependent, see study charts	At 10 µg, equivalent to 7,900 eight-hour work days at 1 µg/m^3 equaling **31 yrs of exposure**. At 20 µg, equivalent to 16,000 eight-hour work days at 1 µg/m^3 equaling **63 yrs of exposure**. At 40 µg, equivalent to 32,000 eight-hour work days at 1 µg/m^3 equaling **126 yrs of exposure**.
Mercer/ 2007	*Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated SWCNTs in a mouse model*	10 µg (Mouse)	dispersed SWCNT are rapidly incorporated into the alveolar interstitium and they produce an increase in collagen deposition	At 10 µg, equivalent to 7,900 eight-hour work days at 1 µg/m^3 equaling **31 yrs of exposure**.
Murray / 2012	*Factoring-in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos*	40 µg (Mouse)	local inflammatory and fibrogenic responses were accompanied by modified systemic immunity, as documented by decreased proliferation of splenic T cells ex vivo on day 28 post exposure.	At 40 µg / m^3, equivalent to 32,000 eight-hour work days at 1 µg/m^3 equaling **126 yrs of exposure**.
Shvedova / 2008	*Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis*	5, 10, 20 µg pharyngeal aspiration study doses (Mouse)	SWCNT inhalation was more effective than aspiration in causing inflammatory response, oxidative stress, collagen deposition, and fibrosis as well as mutations of K-ras gene locus	At 5 µg/m^3, equivalent to 4,000 eight-hour work days at 1 µg/m^3 equaling **16 yrs of exposure**. At 10 µg/m^3, equivalent to 7,900 eight-hour work days at 1 µg/m^3 equaling **31 yrs of exposure**.

				At 20 µg/m^3, equivalent to 16,000 eight-hour work days at 1 µg/m^3 equaling **63 yrs of exposure**.
Shvedova / 2013	*Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: one year postexposure comparisons*	40 µg (Mouse)	SWCNT were the most fibrogenic of these three particles (CNF & asbestos). SWCNT induced cytogenetic alterations seen as micronuclei formation and nuclear protrusions in vivo. Inhalation exposure to SWCNT showed significantly greater inflammatory, fibrotic, and genotoxic effects than bolus pharyngeal aspiration; oncogene mutations	At 40 µg/m^3, equivalent to 32,000 eight-hour work days at 1 µg/m^3 equaling **126 yrs of exposure**.
Mangum / 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*	2 mg/kg (Rat)	*SWCNT do not cause lung inflammation and yet induce the formation of small, focal interstitial fibrotic lesions in the alveolar region of the lungs of rats* (Quote from Abstract)	At 2,000 µg/m^3, equivalent to 42,000 eight-hour work days at 1 µg/m^3 **equaling 167 years of exposure**.
INHALATION				
Shvedova / 2008	*Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis*	5mg/m3 (Mouse)	SWCNT inhalation was more effective than aspiration in causing inflammatory response, oxidative stress, collagen deposition, and fibrosis as well as mutations of K-ras gene locus	Dose was 5000x NISOH REL for 5 hours/day for 4 days. At 5,000 µg/m^3, equivalent to 190,000 eight-hour work days at 1 µg/m^3 equaling **730 yrs of exposure**.

Morimoto / 2012	*Pulmonary toxicity of well-dispersed single-wall carbon nanotubes after inhalation*	0.03 & 0.13 mg/m3 (Rat)	no increases of total cell or neutrophil counts in the bronchoalveolar lavage fluid (BALF), or the concentration of cytokine-induced neutrophil chemoattractant in the lungs or BALF in both the high and low concentration-exposed groups. Pulmonary infiltration of neutrophils was not observed in either exposed group throughout the observation period. Well-dispersed SWCNT did not induce neutrophil inflammation in the lung	Dose was 30x and 130x NISOH REL for 6 hours/day for 20 days. At 0.03 mg/m^3, equivalent to 960 eight-hour work days at 1 µg/m^3 equaling **4 yrs of exposure**. At 0.13 mg/m^3, equivalent to 4100 eight-hour work days at 1 µg/m^3 equaling **16 yrs of exposure**.