Endpoints of Concern for Existing Higher Hazard Substances				
HHS	CANCER	TLV	LD50/LC50	Rationale
Trichloroethylene	IARC 1*	10 ppm	2402 mg/kg oral, mouse	* IARC 2A at time of recommendation causes eye, skin, liver and central nervous system damage and low TLV
CD/CD Comp	IARC 1	.01 mg/m3	2330 mg/kg oral, rat	IARC 1
Perchloroethylene	IARC 2A*	25 ppm	2629 mg/kg oral, rat	*IARC 2B at time of recommendation causes eye, skin, liver and central nervous system damage
Formaldehyde	IARC 1*	0.1 ppm	42 mg/kg oral, mouse	*IARC 2A at time of recommendation reactive, irritating, acutely toxic
Dichloromethane	IARC 2A*	50 ppm	1600 mg/kg oral, rat	*IARC 2B at time of recommendation asphyxiant
Hexavalent Chrome	IARC 1	.0002 mg/m3	80mg/kg oral, rat	IARC 1, Acutely toxic
Dimethylformamide	IARC 2B	5 ppm	2800 mg/kg oral, rat	evidence of testicular cancer in humans, highly mobile in soil
Hydrogen fluoride		.5 ppm	LC50 500 ppm (inh mouse)	acutely toxic, poor warning properties
Cyanide Compounds		No TWA	*LD50 3 mg CN^-/kg oral, rat	acutely toxic, CNS effects
N-propyl bromide	IARC 2B	.1 ppm	2530 mg/kg IP, mouse	NTP reasonably anticipated carcinogen and neurotoxicity
Toluene Diisocyanates (combined)	IARC 2B	.001 ppm	1950 mg/kg oral, mouse	Irritating to eyes, nose, skin and low TLV, IARC 2B, sensitizer

Table shows only key endpoints of concern at time of listing, not all hazards associated with the chemical.

Sources: *Cancer: IARC; TLV ACGIH; LD50/LC50: PubChem; Rationale: Categorization document, SAB Minutes, policy analyses.*
**ATSDR Toxicological Profile for Cyanide: LD50 for Sodium Cyanide*

Endpoints of Concern for Carbon Nanotubes and Fibers					
CNT/F	Cancer	TLV	LD50	Biopersistence	Rationale
MWCNT	IARC 2B* Mesothelioma (Huang 2020, Rittenhausen 2014, Xu 2012, Numano 2019, Wang 2020, Suzui 2016)	1 µg/m3 TWA	> 2,000 mg/kg Oral, rat	Biopersistence (Knudsen 2019, Shinohara 2015, Oyabu 2011, Sato 2013)	Pulmonary toxicity, biopersistence, lung cancer, mesothelioma, and environmental persistence
SWCNT	No data	1 µg/m3 TWA	> 50 mg/kg Oral, rat	Biopersistence (Honda 2017, Shvedova 2013, Wang 2013, Galassi 2020, Quin 2023, Principi 2013)	Pulmonary toxicity and environmental persistence
CNF	No data	1 µg/m3 TWA	>2,000 mg/kg Oral, rat		Pulmonary toxicity

Sources: Nanomaterials GreenScreens – ECHA 2021a, OECD 2016, WHO 2017

Abstracts for CNT/F Mesothelioma and Biopersistence Endpoints		
Author	**Title**	**Abstract**
MWCNT Mesothelioma and Biopersistence		
Huang 2020	Role of inflammation in the malignant transformation of pleural mesothelial cells induced by multi-walled carbon nanotubes	We established an *in vitro* system by co-culturing macrophages and mesothelial cells and exposing these cells to high aspect ratio MWCNTs (0.1 µg/mL) for three months. Results indicated that IL-1β, secreted by macrophages stimulated by MWCNTs, may significantly enhance the release of inflammatory cytokines, such as IL-8, TNF-α, and IL-6, from mesothelial cells. Results obtained from proliferation, migration, invasion, colony formation, and chromosomal aberration studies indicated that MWCNTs may promote malignant transformation of mesothelial cells after long-term and low-dose exposure *via* inflammation. Furthermore, the obtained results demonstrated that the NF-κB/IL-6/STAT3 pathway was active in the malignant transformation of Met 5A cells, induced by MWCNTs, and played an important role in the process. In conclusion, our results showed that the NF-κB (p65)/IL-6/STAT3 molecular pathway, which was mediated by inflammation, played an important role in the malignant transformation of pleural mesothelial cells induced by MWCNTs.
Rittinghausen 2014	The carcinogenic effect of various multi-walled carbon nanotubes (MWCNTs) after intraperitoneal injection in rats	Treatments induced tumors in all dose groups, but incidences and times to tumor differed between groups. Most tumors were histologically and immunohistochemically classified as malignant **mesotheliomas**, revealing a predominantly superficial spread on the serosal surface of the abdominal cavity. Furthermore, most tumors showed invasion of peritoneal organs, especially the diaphragm. All tested MWCNT types caused **mesotheliomas**. We observed highest frequencies and earliest appearances after treatment with the rather straight MWCNT types A and B. In the MWCNT C groups, first appearances of morbid **mesothelioma**-bearing rats were only slightly later. Later during the two-year study, we found **mesotheliomas** also in rats treated with MWCNT D - the most curved type of **nanotubes**. Malignant **mesotheliomas** induced by intraperitoneal injection of different MWCNTs and of asbestos were histopathologically and immunohistochemically similar, also compared with **mesotheliomas** in man, suggesting similar pathogenesis.

Xu 2012	Multi-walled carbon nanotubes translocate into the pleural cavity and induce visceral mesothelial proliferation in rats	We investigated whether **carbon nanotubes** administered into the lung through the trachea induce mesothelial lesions. Male F344 rats were treated with 0.5 mL of 500 μg/mL suspensions of **multi-walled carbon nanotubes** or crocidolite five times over a 9-day period by intrapulmonary spraying. Pleural cavity lavage fluid, lung and chest wall were then collected. **Multi-walled carbon nanotubes** and crocidolite were found mainly in alveolar macrophages and mediastinal lymph nodes. Importantly, the fibers were also found in the cell pellets of the pleural cavity lavage, mostly in macrophages. Both **multi-walled carbon nanotube** and crocidolite treatment induced hyperplastic proliferative lesions of the visceral mesothelium, with their proliferating cell nuclear antigen indices approximately 10-fold that of the vehicle control. The hyperplastic lesions were associated with inflammatory cell infiltration and inflammation-induced fibrotic lesions of the pleural tissues. The fibers were not found in the mesothelial proliferative lesions themselves. In the pleural cavity, abundant inflammatory cell infiltration, mainly composed of macrophages, was observed. Conditioned cell culture media of macrophages treated with **multi-walled carbon nanotubes** and crocidolite and the supernatants of pleural cavity lavage fluid from the dosed rats increased mesothelial cell proliferation in vitro, suggesting that mesothelial proliferative lesions were induced by inflammatory events in the lung and pleural cavity and likely mediated by macrophages. In conclusion, intrapulmonary administration of **multi-walled carbon nanotubes**, like asbestos, induced mesothelial proliferation potentially associated with **mesothelioma** development.
Numano 2019	MWCNT-7 administered to the lung by intratracheal instillation induces development of pleural mesothelioma in F344 rats	**Multi-walled carbon nanotube**-7 (MWCNT-7) fibers are biopersistent and have a structure similar to asbestos. MWCNT-7 has been shown to induce malignant **mesothelioma** when administered by intrascrotal or intraperitoneal injection in rats and mice, and an inhalation study demonstrated that rats exposed to respirable MWCNT-7 developed lung tumors. MWCNT-N, which is similar to MWCNT-7, was shown to induce both lung tumors and malignant **mesothelioma** in rats when administered by trans-tracheal intrapulmonary spraying (TIPS). The present study was performed to investigate the carcinogenicity of MWCNT-7 when administered by the TIPS method. Ten-week-old male F344/Crj rats were divided into 3 groups and administered 0.5 mL vehicle, 0.250 μg/mL MWCNT-7 or 0.250 μg/mL crocidolite once a week for 12 weeks (total doses of 1.5 mg/rat) and then observed for up to 104 weeks. Rats in the MWCNT-7 group began to die from pathologies associated with the development of malignant **mesothelioma** 35 weeks after the final TIPS administration. Overall, the incidence of malignant **mesothelioma** in the MWCNT-7 group was significantly higher than in the vehicle or crocidolite groups.
Wang 2020	Pleural translocation and lesions by pulmonary exposed multi-walled carbon nanotubes	The shape of some types of CNTs is similar to asbestos fibers, which suggests that these CNTs may cause characteristic pleural diseases similar to those found in asbestos-exposed humans, such as pleural plaques and malignant **mesothelioma**. Experimental data indicate that CNTs can induce lung and pleural lesions, inflammation, pleural fibrosis, lung tumors, and malignant **mesothelioma** upon inhalation in the experimental animals. In this review, we focus on the potential of MWCNTs to induce diseases similar to those by asbestos, molecular and cellular mechanisms associated with these diseases, and we discuss a method for evaluating the pleural toxicity of MWCNTs.
Knudsen 2019	Physicochemical predictors of Multi-Walled Carbon Nanotube–induced pulmonary histopathology and toxicity one year after pulmonary deposition of 11 different Multi-Walled Carbon Nanotubes in mice	We have evaluated histological changes in lung tissue 1 year after a single intratracheal instillation of 11 well-characterized MWCNT in female C57BL/6N BomTac mice. Genotoxicity in liver and spleen was evaluated by the comet assay. The dose of 54 μg MWCNT corresponds to three times the estimated dose accumulated during a work life at a NIOSH recommended exposure limit (0.001 mg/m3). Short and thin MWCNT were observed as agglomerates in lung tissue 1 year after exposure, whereas thicker and longer MWCNT were detected as single fibres, suggesting **biopersistence** of both types of MWCNT. The thin and entangled MWCNT induced varying degree of pulmonary inflammation, in terms of lymphocytic aggregates, granulomas and macrophage infiltration, whereas two thick and straight MWCNT did not. By multiple regression analysis, larger diameter and higher content of iron predicted less histopathological changes, whereas higher cobalt content significantly predicted more histopathological changes. No MWCNT-related fibrosis or tumours in the lungs or pleura was found. One thin and entangled MWCNT induced increased levels of DNA strand breaks in liver; however, no physicochemical properties could be related to genotoxicity. This study reveals physicochemical-dependent difference in MWCNT-induced long-term, pulmonary histopathological changes. Identification of diameter size and cobalt content as important for MWCNT toxicity provides clues for designing MWCNT, which cause reduced human health effects following pulmonary exposure.

Shinohara 2015	Long-term retention of pristine multi-walled carbon nanotubes in rat lungs after intratracheal instillation	We administered pristine MWCNTs well dispersed in 0.5 mg ml1 Triton-X solution to rats at doses of 0.20 or 0.55 mg via intratracheal instillation and investigated clearance over a 12-month observation period. The pristine MWCNTs pulmonary burden was determined 1, 3, 7, 28, 91, 175 and 364 days after instillation using a method involving combustive oxidation and infrared analysis, combined with acid digestion and heat pretreatment. As 0.15- and 0.38-mg MWCNTs were detected 1 day after administration of 0.20 and 0.55 mg MWCNTs, respectively, approximately 30% of administrated MWCNTs may have been cleared by bronchial ciliary motion within 24 h of administration. After that, the pulmonary MWCNT burden did not decrease significantly over time for up to 364 days after instillation, suggesting that MWCNTs were not readily cleared from the lung. Transmission electron microscopy (TEM) showed that alveolar macrophages internalized the MWCNTs and retained in the lung for at least 364 days after instillation. MWCNTs were not detected in the liver or brain within the 364-day study period (< 0.006 mg per brain).
Suzui 2016	Multiwalled carbon nanotubes intratracheally instilled into the rat lung induce development of pleural malignant mesothelioma and lung tumors	MWCNT-N was fractionated by passing it through a sieve with a pore size of 25 µm. The average lengths of the MWCNT were 4.2 µm before filtration and 2.6 µm in the flow-through fraction; the length of the retained MWCNT could not be determined. For the present study, 10-week-old F344/Crj male rats were divided into five groups: no treatment, vehicle control, MWCNT-N before filtration, MWCNT-N flow-through and MWCNT-N retained groups. Administration was by the trans-tracheal intrapulmonary spraying (TIPS) method. Rats were administered a total of 1 mg/rat during the initial 2 weeks of the experiment and then observed up to 109 weeks. The incidences of malignant mesothelioma and lung tumors (bronchiolo-alveolar adenomas and carcinomas) were 6/38 and 14/38, respectively, in the three groups administered MWCNT and 0/28 and 0/28, respectively, in the control groups. All malignant mesotheliomas were localized in the pericardial pleural cavity.
Oyabu 2011	Biopersistence of inhaled MWCNT in rat lungs in a 4-week well-characterized exposure	We conducted an inhalation study of a multi-wall **carbon** nanotube (MWCNT) as a hazard assessment. Male Wistar rats were exposed to well-dispersed MWCNT for 4 weeks by whole body inhalation. The exposure concentration in the chamber was 0.37 ± 0.18 mg/m^3. About 70% of the MWCNTs in the chamber were single fiber. The geometric mean diameter (geometric standard deviation, GSD) and geometric mean length (GSD) of the aerosolized MWCNTs in the chamber were 63 nm (1.5) and 1.1 µm (2.7), respectively. The amounts of MWCNT deposited in the rat lungs were determined by the X-ray diffraction method and elemental **carbon** analysis. The average deposited amounts at 3 days after the inhalation were 68 µg/lung by the X-ray diffraction method and 76 µg/lung by elemental **carbon** analysis. The calculated deposition fractions were 18% and 20% in each analysis. The amount of retained MWCNT in the lungs until 3 months after the inhalation decreased exponentially and the calculated biological half times of MWCNT were 51 days and 54 days, respectively. The clearance was not delayed, but a slight increase in lung weight at 3 days after the inhalation was observed.
Sato 2013	Long-term biopersistence of tangled oxidized carbon nanotubes inside and outside macrophages in rat subcutaneous tissue	Tangled oxidized multi-walled CNTs (t-ox-MWCNTs) were implanted into rat subcutaneous tissues and structural changes in the t-ox-MWCNTs located inside and outside of macrophages were studied for 2 years post-implantation. The majority of the large agglomerates were present in the intercellular space, maintained a layered structure, and did not undergo degradation. By contrast, small agglomerates were found inside macrophages, where they were gradually degraded in lysosomes. None of the rats displayed symptoms of cancer or severe inflammatory reactions such as necrosis. These results indicate that t-ox-MWCNTs have high **biopersistence** and do not evoke adverse events in rat subcutaneous tissue in vivo, demonstrating their potential utility as implantable biomaterials.
SWCNT Biopersistence		
Honda 2017	A 104-week pulmonary toxicity assessment of long and short single-wall carbon nanotubes after a single intratracheal instillation in rats	We compared long-term pulmonary toxicities after a single intratracheal instillation of two types of dispersed single-wall carbon nanotubes (SWCNTs), namely, those with relatively long or short linear shapes with average lengths of 8.6 and 0.55 mm, respectively. Both types of SWCNTs were instilled intratracheally in male F344 rats at 0.2 or 1.0 mg/kg (long SWCNTs) or 1.0 mg/kg (short SWCNTs). Pulmonary responses were characterized at 26, 52 and 104 weeks after a single instillation. 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. Both bronchiolo-alveolar adenoma and carcinoma were found in the negative-control group, the high-dose long-nanotube group, and the short-nanotube group at 104 weeks post-instillation, although the incidences were not statistically different. The genotoxicity of the SWCNTs was also evaluated by performing in vivo comet assays with lung cells obtained 26 weeks post-instillation. No significant changes in the percent tail deoxyribonucleic acid were found in any group. These findings suggested that most long SWCNTs were deposited at the terminal bronchioles and that a considerable amount of short SWCNTs reached the alveolus, resulting in chronic inflammatory responses, but no genotoxicity in the lungs.
Shvedova 2013	Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: one year postexposure comparisons	We compared inflammatory, fibrogenic, and genotoxic effects of CNF, SWCNT, or asbestos in mice 1 yr after pharyngeal aspiration. In addition, we compared pulmonary responses to SWCNT by bolus dosing through pharyngeal aspiration and inhalation 5 h/day for 4 days, to evaluate the effect of dose rate. The aspiration studies showed that these particles can be visualized in the lung at 1 yr postexposure, whereas some translocate to lymphatics. All these particles induced chronic bronchopneumonia and lymphadenitis, accompanied by pulmonary fibrosis. CNF and asbestos were found to promote the greatest degree of inflammation, followed by SWCNT, whereas SWCNT were the most fibrogenic of these three particles. Furthermore, SWCNT induced cytogenetic alterations seen as micronuclei formation and nuclear protrusions in vivo. Importantly, inhalation exposure to SWCNT showed significantly greater inflammatory, fibrotic, and genotoxic effects than bolus pharyngeal aspiration. Finally, SWCNT and CNF, but not asbestos exposures, increased the incidence of K-ras oncogene mutations in the lung. No increased lung tumor incidence occurred after 1 yr postexposure to SWCNT, CNF, and asbestos. Overall, our data suggest that long-term pulmonary toxicity of SWCNT, CNF, and asbestos is defined, not only by their chemical composition, but also by the specific surface area and type of exposure.
Wang 2013	Neoplastic-like transformation effect of single-walled and multi-walled carbon nanotubes compared to asbestos on human lung small airway epithelial cells	Accumulating evidence indicates that carbon nanotubes (CNTs) are biopersistent and can cause lung damage. With similar fibrous morphology and mode of exposure to asbestos, a known human carcinogen, growing concern has arisen for elevated risk of CNT-induced lung carcinogenesis; however, relatively little is known about the long-term carcinogenic effect of CNT. Neoplastic transformation is a key early event leading to carcinogenesis. We studied the ability of single- and multi-walled CNTs to induce neoplastic transformation of human lung epithelial cells compared to asbestos. Long-term (6-month) exposure of the cells to occupationally relevant concentrations of CNT in culture caused a neoplastic-like transformation phenotype as demonstrated by increased cell proliferation, anchorage-independent growth, invasion and angiogenesis. Whole-genome expression signature and protein expression analyses showed that single- and multi-walled CNTs shared similar signaling signatures which were distinct from asbestos. These results provide novel toxicogenomic information and suggest distinct particle-associated mechanisms of neoplasia promotion induced by CNTs and asbestos.
Galassi 2020	Long-term *in vivo* biocompatibility of single-walled carbon nanotubes	In this work, we investigated the short and long term biodistribution and biocompatibility of a purified DNA-encapsulated single-walled carbon nanotube complex consisting of an individual nanotube chirality, administered intravenously. Bulk biodistribution measurements in mice found that, consistent with previous studies on similar complexes, the nanotubes localized predominantly to the liver. Using near-infrared hyperspectral microscopy to image single nanotube complexes, nanotube complexes were found in other organs such as the kidney, spleen, hearts, and lungs and persisted in some organs at for up to 5 months.
Qin 2023	Long-term intravenous administration of carboxylated single-walled carbon nanotubes induces persistent accumulation in the lungs and pulmonary fibrosis via the nuclear factor-kappa B pathway	Long-term intravenous injection of c-SWNTs caused sustained embolization in lung capillaries and granuloma formation. It also induced a persistent inflammatory response that was regulated by the nuclear factor-kappa B signaling pathway, and which resulted in pulmonary fibrogenesis. c-SWNTs trapped within lung capillaries traversed capillary walls and injured alveolar epithelial cells, thereby stimulating production of pro-inflammatory cytokines (tumor necrosis factor-alpha and interleukin-1 beta) and pro-fibrotic growth factors (transforming growth factor-beta 1). Protein levels of type-I and type-III collagens, matrix metalloproteinase-2, and the tissue inhibitor of metalloproteinase-2 were upregulated after intravenous exposure to c-SWNTs as determined by immunohistochemical assays and Western blotting, which suggested collagen deposition and remodeling of the extracellular matrix.
Principi 2016	Systemic distribution of single-walled carbon nanotubes in a novel	Using an in vivo model for CNT environmental exposure, mimicking CNT exposition at the workplace, we previously found that CNTs rapidly enter and disseminate in the organism, initially accumulating in the lungs and brain and later

	model: alteration of biochemical parameters, metabolic functions, liver accumulation, and inflammation in vivo	reaching the liver and kidneys via the bloodstream in CD1 mice. Here, we monitored and traced the accumulation of single-walled CNTs (SWCNTs), administered systemically in mice, in different organs and the subsequent biological responses. Using the novel in vivo model, MITO-Luc bioluminescence reporter mice, we found that SWCNTs induce systemic cell proliferation, indicating a dynamic response of cells of both bone marrow and the immune system. We then examined metabolic (water/food consumption and dejections), functional (serum enzymes), and morphological (organs and tissues) alterations in CD1 mice treated with SWCNTs, using metabolic cages, performing serum analyses, and applying histological, immunohistochemical, and ultrastructural (transmission electron microscopy) methods. We observed a transient accumulation of SWCNTs in the lungs, spleen, and kidneys of CD1 mice exposed to SWCNTs. A dose- and time-dependent accumulation was found in the liver, associated with increases in levels of aspartate aminotransferase, alanine aminotransferase and bilirubinemia, which are metabolic markers associated with liver damage. Our data suggest that hepatic accumulation of SWCNTs associated with liver damage results in an M1 macrophage-driven inflammation.