SINGLE WALLED CARBON NANOTUBES (SWCNTs)

(CAS #308068-56-6)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: June 10, 2021

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[1] Although CPA's Assessment Expiration Policy (CPA 2018a) indicates that Benchmark 1 assessments have no expiration date, ToxServices strives to review BM-1s in a five-year period to ensure currency of data presented in the BM-1 GreenScreen® assessments.

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GreenScreen® Executive Summary for Single Walled Carbon Nanotubes (SWCNTs)

Carbon nanotubes (CNT) are a group of nanomaterials consisting of only one or several hexagonal graphite sheets of carbon atoms rolled into tubes. They are black crystalline particles that are highly insoluble in water due to their graphitic structure. CNTs have very high aspect ratios with diameters lower than about 100 nm (0.000001 mm) and lengths that can reach several hundred micrometers. CNTs fall into two classes: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Despite the obvious commonality, SWCNTs and MWCNTs have significantly different physical properties from each other because of their structural differences. The CAS number of 308068-56-6 is assigned to carbon nanotubes in general regardless of the types (single, double or multi wall).

SWCNTs consist of one layer of graphene cylinder of 1 – 2 nm in diameter and typically 1 – 10 μm in length and aspect ratio up to 10,000. The length of a C–C bond in a graphene sheet of SWCNT is 0.142 nm. SWCNTs do not normally exist as individual tubes. Due to van der Waals forces, SWCNTs tend to form agglomerates or aggregates leading to the construction of microscopic bundles or ropes which can reach 5–50 nm in diameter. Due to differences in manufacturing processes, SWCNTs can vary widely with respect to their form (tube length and diameter), particle size, specific surface area and residual impurities and, consequently, they might exert quite different toxic effects. Therefore, in this GreenScreen® assessment, ToxServices considered six types of SWCNTs that were either reviewed by the Organization for Economic Co-operation and Development (OECD) or registered under REACH with an EC number of 943-098-9 and a trade name of Tuball™ SWCNT.

SWCNTs were assigned a **GreenScreen Benchmark™ Score of 1** (**"**Avoid—Chemical of High Concern"). This score is based on the following hazard score combinations:

- Benchmark 1b
    - Very High persistence-P + High Group II* Human Toxicity (systemic toxicity repeated exposure-STr*)

Data gaps (DG) exist for carcinogenicity-C and endocrine activity-E[2]. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), SWCNTs meet requirements for a GreenScreen Benchmark™ Score of 1 despite the hazard data gaps. In a worst-case scenario, if SWCNTs were assigned a High score for the data gaps C or E, it would still be categorized as a Benchmark 1 Chemical.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* tests for genotoxicity, skin irritation and eye irritation. The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties:

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

No Type I (input data) uncertainties on using SWCNTs' NAMs dataset are identified. SWCNTs' Type II (extrapolation output) uncertainties include the limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, the non-applicability of the bacterial reverse mutation test to nanomaterials, the limitation of *in vitro* skin irritation test **(**RHE, OECD Guideline 439) to identify substances classified

[2] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen® Guidance v1.4 Annex 2.

as mild skin irritant (GHS Category 3), and the limitation of the *in vitro* eye irritation test **(**RhCE test, OECD Guideline 492) to differentiate between Category 2 and Category 1, or between Category 2A and Category 2B. The type II errors can be alleviated by the use of genotoxicity test batteries *and in vivo* data for skin and eye irritation as there are no validated *in vitro* methods available for the direct identification of Category 2 eye irritants and Category 3 skin irritants.

GreenScreen® Hazard Summary Table for SWCNTs

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	**M**	**R**	**D**	**E**	**AT**	**ST**		**N**		**SnS**	**SnR**	**IrS**	**IrE**	**AA**	**CA**	**P**	**B**	**Rx**	**F**
						s	r*	s	r*	*	*								
DG	*M*	*L*	*L*	DG	*L*		*H*		**L**	**L**	*L*	*L*	*H*	**L**	*H*	*vH*	**vL**	*L*	*L*

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Chemical Assessment for Single Walled Carbon Nanotubes (SWCNTs) (CAS #308068-56-6)

Method Version: GreenScreen® Version 1.4
Assessment Type[3]: Certified
Assessor Type: Licensed GreenScreen® Profiler

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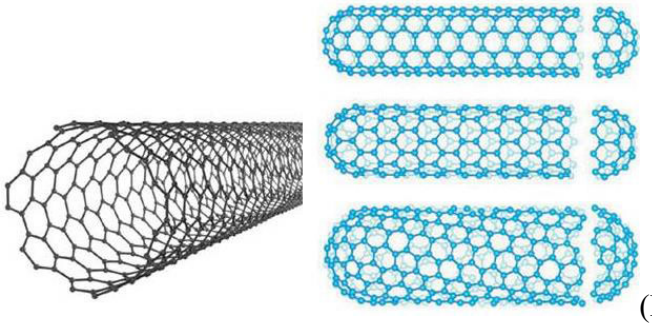
Date: June 10, 2021

ToxServices Review Date: June 10, 2026[4]

Chemical Name: Single walled carbon nanotubes (SWCNTs)

CAS Number: 308068-56-6[5]

Chemical Structure(s): Carbon nanotubes (CNT) are a group of nanomaterials consisting of only one or several hexagonal graphite sheets of carbon atoms rolled into tubes. Single walled carbon nanotubes (SWCNT) consist of one layer of graphene cylinder of 1 – 2 nm in diameter and typically 1 – 10 µm in length and aspect ratio up to 10,000 (ECHA 2021a, IARC 2017). SWCNTs do not normally exist as individual tubes. Due to the van der Waals forces, they tend to form agglomerates or aggregates leading to the construction of microscopic bundles or ropes which can reach 5–50 nm in diameter. These bundles tend to agglomerate loosely into small clumps (IARC 2017). The following depicts an exemplary sample of a SWCNT:



(ECHA 2021a)

Also called: Carbon nanotubes, Fullerenes, tubular, Tubular fullerenes, Tubulenes (Pharos 2021).

[3] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[4] Although CPA's Assessment Expiration Policy (CPA 2018a) indicates that Benchmark 1 assessments have no expiration date, ToxServices strives to review BM-1s in a five-year period to ensure currency of data presented in the BM-1 GreenScreen® assessments.
[5] The CAS number of 308068-56-6 is assigned to carbon nanotubes in general regardless of the types (single, double or multi wall). The EC number of 943-098-9 is associated with SWCNT.

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
No surrogates are used in this assessment. However, due to differences in manufacturing processes, SWCNTs can vary widely with respect to their form (tube length and diameter), particle size, specific surface area and residual impurities and, consequently, they might exert quite different toxic effects. Therefore, to properly interpret and assess their observed toxic effects, the SWCNTs used in each individual study should be characterized in detail with respect to all of the physical and chemical properties that might have biological relevance, including the possible presence of impurities such as metals. The Organization for Economic Co-operation and Development (OECD) Group evaluated the safety of SWCNTs and conducted a series of toxicological tests on SWCNTs manufactured by different suppliers (OECD 2016). Most tests were conducted on two types of SWCNTs, Nikkiso SWCNT and Super Growth SWCNT. For the carcinogenicity endpoint, data were available for AIST SWCNT and Shenzhen Nanotech SWCNT. For the genotoxicity, testing was also performed on CNI SWCNT. ToxServices considered all these five types of SWCNTs that were reviewed by the OECD Group in this GreenScreen® assessment. In addition, ToxServices identified Tuball™ SWCNT as another representative substance to this category which is registered under REACH with an EC number of 943-098-9 (ECHA 2021a). The physicochemical characterization data for the six types of SWCNTs used in this assessment are listed below:

1. Nikkiso SWCNT: Contains 4% of iron and very small amounts of other metallic impurities and is characterized with a tube diameter of 3.03 nm, a particle size diameter of 2.7 µm and a specific surface area of 878 m^2/g. It is insoluble in water (OECD 2016, WHO 2017).

2. Super Growth SWCNT: Contains > 99% carbon and very small amounts of other metallic impurities and is characterized with a tube diameter of 1.86 nm, a particle size diameter of 8.2 nm, a length of 0.23 µm, a pour density of 0.0192 g/cm^3, and a specific surface area of 1,064 m^2/g. It is insoluble in water (OECD 2016, WHO 2017).

3. AIST SWCNT: It is synthesized by the National Institute of Advanced Industrial Science and Technology (AIST) of Japan and contains 145 ppm iron, 103 ppm nickel, 34 ppm chromium, 2 ppm manganese, and 12 ppm aluminum. It is characterized with a primary particle maximum length of 1,200 µm; primary particle diameter of 3.0 nm; and aggregate length of 0.32 µm, aggregate diameter of 12.0 nm (WHO 2017).

4. Shenzhen Nanotech SWCNT: It is characterized with a tube diameter < 2 nm, a length of 4-15 µm, and 90% pure (WHO 2017).

5. CNI SWCNT. It is synthesized by Carbon Nanotechnologies, Inc (CNI) using high-pressure catalytic CO conversion (HiPco method). It is characterized with a tube diameter of 1.0 ± 0.2 nm and several hundred nanometers to several micrometers long; mass medium aerodynamic diameter of 4.2 µm; diameter 1–4 nm; length 0.5–1 µm; surface area 1,040 m^2/g (WHO 2017).

6. Tuball™ SWCNT: Contains carbon black and iron oxide as impurities. It is characterized with a tube diameter of 1.8 nm and length from 1.3 µm to 18 µm (median = 4.8 µm) (ECHA 2021a).

Identify Applications/Functional Uses:
Used in composite materials as a method of improving mechanical strength (IARC 2017).

Known Impurities[6]:
Due to differences in manufacturing processes, SWCNTs may contain a variety of residual impurities such as multi walled carbon nanotubes (MWCNT), fullerene, amorphous carbon, graphite, and catalytic metals such as iron, nickel, cobalt, and molybdenum (IARC 2017). Nikkiso SWCNT contains 4% of iron and very small amounts of other metallic impurities. Super Growth SWCNT contains > 99% carbon and very small amounts of other metallic impurities. Tuball™ SWCNT contains carbon black and iron oxide as impurities.

GreenScreen® Summary Rating for SWCNTs[7,8 9,10]: SWCNTs were assigned a **GreenScreen Benchmark™ Score of 1 (**"Avoid—Chemical of High Concern") (CPA 2018b). This score is based on the following hazard score combinations:

- Benchmark 1b (vPT)
    - Very High Persistence (P) + Very High Group II* Human Toxicity (systemic toxicity repeated exposure – STr*)

A data gap (DG) exists for endocrine activity-E**.** As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis) (CPA 2018b), SWCNTs meet requirements for a GreenScreen Benchmark™ Score of 1 despite the hazard data gap. In a worst-case scenario, if SWCNTs were assigned a High score for the data gap E, it would still be categorized as a Benchmark 1 Chemical.

Figure 1: GreenScreen® Hazard Summary Table for SWCNTs

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
DG	*M*	*L*	*L*	DG	*L*		*H*		**L**	**L**	*L*	*L*	*H*	**L**	*H*	*vH*	**vL**	*L*	*L*

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

Environmental Transformation Products
No transformation products are identified as SWCNTs are inorganic nanomaterials that are persistent in the environment.

[6] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.
[7] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.
[8] See Appendix A for a glossary of hazard endpoint acronyms.
[9] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[10] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen® Guidance v1.4 Annex 2.

Introduction

CNTs are a group of nanomaterials consisting of only one or several hexagonal graphite sheets of carbon atoms rolled into tubes. They have very high aspect ratios with diameters lower than about 100 nm (0.000001 mm) and lengths that can reach several hundred micrometers. CNTs fundamentally fall into two classes: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Despite the obvious commonality, SWCNTs and MWCNTs have significantly different physical properties from each other because of their structural differences. The most important feature that distinguishes SWCNTs is that the wall of the nanotube consists of the only one graphene layer. The length of a C–C bond in a graphene sheet of SWCNT is 0.142 nm. The CAS number of 308068-56-6 is assigned to carbon nanotubes in general regardless of the types (single, double or multi wall) (IARC 2017).

ToxServices assessed SWCNTs against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2020).

U.S. EPA Safer Choice Program's Safer Chemical Ingredients List

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2020a). It can be accessed at: http://www2.epa.gov/saferchoice/safer-ingredients. Chemicals on the SCIL have been assessed for compliance with the Safer Choice Standard and Criteria for Safer Chemical Ingredients (U.S. EPA 2015).

SWCNTs were not listed on the SCP SCIL.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2021) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[11] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for SWCNTs can be found in Appendix C.

- SWCNTs are listed as LT-P1 chemicals when screened using Pharos, and therefore a full GreenScreen® is required.
- SWCNTs are not listed on the U.S. DOT list.
- SWCNTs are on the following list for multiple endpoints. Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.
    - ChemSec – SIN List - CMR - Carcinogen, Mutagen &/or Reproductive Toxicant

Hazard Statement and Occupational Control

SWCNTs are associated with two Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements as shown in Table 1, identified by the Australia government (Pharos 2021). In addition, Tuball™ SWCNT with an EC number of 943-098-9 is associated with one GHS hazard statement identified by the majority of notifiers in the ECHA classification and labeling inventory (C&L) and by the authors of its REACH registration dossier (ECHA 2021a,b). General

[11] DOT lists are not required lists for GreenScreen® List Translator v1.4. They are reference lists only.

personal protective equipment (PPE) recommendations are presented in Table 2 below. No occupational exposure limits (OEH) were identified.

Table 1: GHS H Statements for SWCNT (CAS #308068-56-6) (Pharos 2021, ECHA 2021b)	
H Statement	**H Statement Details**
H351	Suspected of causing cancer (SWCNTs)
H373	May cause damage to organs through prolonged or repeated exposure (SWCNTs)
H319	Causes serious eye irritation (Tuball™ SWCNT)

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for SWCNTs (CAS #308068-56-6)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
Wear eye protection, protective gloves, protective clothing, respiratory protection	OCSiAl 2020	None	

Physicochemical Properties of SWCNTs

CNTs are black crystalline particles that are highly insoluble in water. They have very high aspect ratios with diameters lower than about 100 nm (0.000001 mm) and lengths that can reach several hundred micrometers (IARC 2017). They may be classified as man-made fibrous materials, according to the World Health Organization (WHO) respirable fiber definition, that is, a particle longer than 5 µm, <3 µm in diameter, and with an aspect ratio of >3:1 (Oberdörster et al. 2015). Due to differences in manufacturing processes, CNTs including SWCNTs can vary widely in their physiochemical properties which may affect their potential toxicity. The most important physicochemical characteristics which influence toxicity of CNTs are: method of generation, shape (length, width, morphology), agglomeration/aggregation, surface properties (area, charge, defects, coating, reactivity), impurities, and density. These properties have been previously described for the six types of SWCNTs used in this assessment. Table 3 lists the other physicochemical for Tuball™ SWCNT. Due to the low density of CNT, it is anticipated that respirable particles may be generated during manufacturing, as a result of transfer, weighing, mixing, and blending of CNT. Therefore, inhalation is considered a primary route for human exposure.

Table 3: Physical and Chemical Properties of SWCNTs (CAS #308068-56-6)		
Property	**Value**	**Reference**
Molecular formula	Cx	ECHA 2021a
SMILES Notation	[C]	ECHA 2021a, Pharos 2021
Molecular weight	Approximately 16	ECHA 2021a, Pharos 2021
Physical state	Solid, nanomaterial form	ECHA 2021a
Appearance	Black powder	ECHA 2021a
Melting point	> 400°Cat 101325 Pa (Tuball®)	ECHA 2021a
Boiling point	> 400°C No boiling point up to 400°C observed.	ECHA 2021a
Vapor pressure	Not conducted as melting point > 400°C	ECHA 2021a
Water solubility	Insoluble in water	OECD 2016, ECHA 2021a
Dissociation constant	Not conducted as the substance does not contain any functional groups that may dissociate.	

Table 3: Physical and Chemical Properties of SWCNTs (CAS #308068-56-6)		
Property	**Value**	**Reference**
Density/specific gravity	1.877 g/cm³ at 20 °C	
Partition coefficient	Not applicable, as substance is inorganic	
Particle size	Thin tube with diameters between 1.0 nm and 2.2 nm and highest intensities near 1.6 nm.	ECHA 2021a
Structure	Tube cylindrical surface is formed by 6-membered rings consisting of carbon atoms linked by a double bond. - Carbon nanotube chirality is random. - Tube length: 1 - 10 µm. - Agglomeration: CNTs tend for form bundles	ECHA 2021a
Bioavailability	SWCNTs are not absorbed through skin and are estimated to have poor systemic absorption through the lungs and gastrointestinal tract.	ECHA 2021a

Toxicokinetics

Measured data were available on the distribution and metabolism of SWCNTs. For absorption and excretion, authors of the REACH registration dossier for Tuball™ SWCNT made predictions using information on the SWCNTs' physicochemical properties.

- Absorption
  - ECHA 2021a
    - *Oral*: Generally, absorption of SWCNT from the intestinal is expected to occur only to a very minor extent (assumed <1%). This is supported by lack of toxicity observed in male and female rats treated with oral doses of Tuball™ SWCNT (>1,000 mg/kg/day) in a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD Guideline 422). As dark colored feces were observed in the high dose group as of the fourth day of dosing, the excretion of non-absorbed material was shown.
    - *Inhalation*: For CNTs, inhalation is considered a primary route for human exposure due to the presence of respirable particles. For Tuball™ SWCNT, absorption via the inhalation route is assumed low (<1%) as the substance is extremely difficult to get airborne as an aerosol. In the attempt to perform a repeated dose subchronic toxicity study via inhalation with Tuball™, it was impossible to create a respirable and stable aerosol/dust atmosphere of the test material, as the SWCNT clogged up each and every equipment that was tried (six different setups were investigated for suitability). Additionally, the dustiness of Tuball™ was measured and found to be more than 1 – 2 orders of magnitude lower than other carbon nanotubes reported in public literature. This confirms that dustiness and ability to form aerosols of Tuball™ is rather limited and thus, inhalation exposure may be assumed to be significantly lower than for other carbon nanomaterials.
    - *Dermal*: No dermal absorption is expected from SWCNTs (< 1%) and it can be assumed that penetration of skin and subsequent dermal exposure is not relevant. This is based on data from the *in vitro* skin corrosion test (OECD 431) and *in vivo* skin sensitization study (Buehler Test, OECD 406 study) performed with Tuball™

SWCNT, which showed no indication of any interaction with reconstructed human epidermis or with guinea pig skin.

- Distribution
  - ECHA 2021a
    - The biodistribution of SWCNTs was investigated in rats using the iodogen oxidative method in which SWCNTs were functionalized with iodine and the iodine labelled SWCNTs (a single dose of 100 µl) was then applied by intratracheal instillation. Animals were sacrificed and investigated 2, 4, 8, 24 and 72 hours after dosing. More than 99% of radioactivity remained in the trachea and based on total radioactivity, decreasing concentrations were found in the other organs/fluids as follows: trachea >> urine > stomach > small intestine > serum > bladder > blood vessel > kidney > liver > lung > adrenal > femoral head > spleen > testis > thymus > thyroid > heart > fat > muscle > brain. The total administered radioactivity was located mainly in the trachea (>99%, supporting low absorption). Compared to the 2 h group, the radioactivity distribution for the 24 h group was significantly increased, and this was not significantly reduced after 72 h. The radioactivity distribution in serum and blood vessels for the 2 h group increased quickly within 8 h without an obvious decrease, but levels were distinctly depressed after 24 h. The low radioactivity detected in the thyroid gland, the major target organ of iodine, indicates that the tracer is stable and only a very small amount of labelled iodine is lost from the SWCNTs. The total recovery of radioactivity in the experiment was about 80%. Based on this, authors concluded that distribution of SWCNTs is assumed to take place mainly via blood and that the substance does not bioaccumulate in tissue. The clearing mechanisms have resulted in highest amounts found in urine, stomach and small intestine. Although, functionalization of SWCNTs by the iodogen oxidative method alters the surface and composition of SWCNT, which may impact its toxicity, this method remains the best test so far, showing that SWCNTs are non-bioaccumulative in rodents.
- Metabolism
  - ECHA 2021a
    - The biodegradation of several types of SWCNTs in tissue has been extensively investigated using *in vitro* and *in vivo* test methods. These studies showed that enzyme catalyzed oxidative pathway is the main mechanism for clearance / metabolism of SWCNTs. Different enzymatic catalytic pathways for the degradation of CNTs have been identified such as horseradish peroxidase (HRP), myeloperoxidase (MPO) and eosinophil peroxidase (EPO) and several oxidative mechanisms have been recognized showing that SWCNTs can be biodegraded by MPO/H_2O_2, MPO/H_2O_2/Cl^-, OCl^- and $ONOO^-$, as well as others. These enzymes are capable to biodegrade SWCNT ultimately to CO_2 and it can be assumed that such mechanisms not only take place in rodents but also in biota such as the aquatic environment.
- Excretion
  - ECHA 2021a
    - In the previously described iodogen oxidative test with SWCNTs, the highest amount of labelled material that was absorbed via inhalation exposure (more than 99% remained unabsorbed in the trachea) was found in urine and thus clearance via urine appears an efficient excretion route for absorbed SWCNTs.

    - The main route for clearance from the body following oral uptake is via feces, as was seen in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD Guideline 422) performed with Tuball™ in which no changes in the consistency of the feces were noted for the male and female animals of the control group and the treatment groups (100, 300 or 1,000 mg /kg/day). However, dark discolored feces were noted continuously from test day 18 (4 days after the start of dosing) until the end of the study for all male and female animals of the high dose group. This was due to the high concentration of the administered test item (black powder) and showed that the main elimination route from the body by oral (via feed) administration is via feces.
- In summary, oral and dermal absorption of SWCNTs is assumed to be low. Inhalation absorption is assumed to be highly likely when the test substance has the ability to form aerosols. If marginal amounts of SWCNT are absorbed or enter body fluids, distribution is assumed to take place mainly via blood. SWCNTs are expected to biodegrade ultimately to CO_2 via an enzyme catalyzed oxidative pathway. The main excretion pathway for absorbed SWCNT is expected to be via urine.

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): DG
SWCNTs were assigned a score of Data Gap for carcinogenicity based on insufficient data available.

- Authoritative and Screening Lists
    - *Authoritative:* IARC Group 3 - Not classifiable as to their carcinogenicity to humans.
    - *Screening:* GHS – Australia: H351 - Suspected of causing cancer.
- WHO 2017, IARC 2017
    - The carcinogenicity of AIST SWCNT was tested in two studies using male SD rats receiving a single intratracheal injection of the SWCNT. In the first experiment, the rats were given a single dose of 1 mL/kg of 0, 0.2, or 2.0 mg/mL solution of SWCNT in Tween 80 in PBS; doses were equivalent to 0.0, 0.2, or 2.0 mg/kg, and six rats per group were killed 24 hours, 3 days, 1 week, 4 weeks, or 13 weeks later. In a second experiment, the rats were given a single dose of 1 mL/kg of a 0, 0.04, 0.2, or 1.0 mg/mL solution of SWCNT in Tween 80 in PBS (doses corresponding to 0.0, 0.4, 0.2, or 1.0 mg/kg), and six rats per group were killed 3 days, 1 week, 4 weeks, 13 weeks, or 26 weeks later. No lung tumors were reported in any group. The International Agency for Research on Cancer (IARC) Working Group noted the short duration of the experiments and judged the study to be inadequate for an evaluation of carcinogenicity.
    - In a 12-month carcinogenicity study, two groups of six F344 rats were injected with a gelatin capsule containing either 10 mg/rat of Shenzhen Nanotech SWCNT or zinc oxide as a negative control. Mesotheliomas were not found but foreign body granulomatous lesions were observed in SWCNT-exposed rats. The IARC Working Group noted the small number of animals, the short duration of the study, that the age and sex of the animals were not reported, and the lack of a vehicle control. The study was judged to be inadequate for an evaluation of carcinogenicity.
    - Based on the results from the above studies, the IARC concluded that there is inadequate evidence in experimental animals for the carcinogenicity of SWCNTs and they are not classifiable as to their carcinogenicity to humans (Group 3). Accordingly, no

carcinogenicity hazard classification was assigned for SWCNTs in the WHO report with the evidence being considered as weak.

- Based on the weight of evidence, a score of Data Gap was assigned. Carcinogenicity studies were available on two types of SWCNAT which showed no signs of tumors. However, these studies were considered inadequate for the classification purposes as the exposure routes used were not relevant for human exposure (e.g., injection in the intrascrotal cavity). The IARC concluded that there is inadequate evidence in experimental animals for the carcinogenicity of SWCNTs and they are not classifiable as to their carcinogenicity to humans (Group 3). IARC Group 3 corresponds to GreenScreen® scores of Low, Moderate or High. SWCNTs are listed by the GHS-Australian screening list as H351 (suspected of causing cancer); which corresponds to a GreenScreen® score of Moderate. The basis of such classification is not reported. SWCNTs are also listed by the ChemSec – SIN screening List as Carcinogen. This is due to the fact that IARC classified a particular type of long and rigid CNT, designated as MWCNT-7, as Group 2B (possibly carcinogenic to humans) on the basis of available animal studies (IARC 2017). The IARC also concluded that there was limited evidence of carcinogenicity for the other types of MWCNTs with dimensions similar to MWCNT-7, and inadequate evidence for SWCNTs. According to IARC, the results of the carcinogenicity studies on CNTs suggest that length, rigidity (based on diameter) and durability of the MWCNT play a key role in the development of mesothelioma; however, due to the limited number of studies available, there are difficulties in determining the minimum physical parameters that would lead to the carcinogenic response. Furthermore, the lack of coherent evidence across the various distinct CNTs precluded generalization to other types of CNTs. Therefore, ToxServices disregarded the ChemSec – SIN listing of SWCNTs as grouping all CNTs into one entry on the SIN List is not scientifically reasonable. In the absence of standard carcinogenicity data on SWCNTs, and the basis of GHS – Australia screening list's classification (Category 2), ToxServices assigned a Data Gap to this endpoint.

Mutagenicity/Genotoxicity (M) Score (H, M, or L): *M*

SWCNTs were assigned a score of Moderate for mutagenicity/genotoxicity based on positive results for DNA damage seen in *in vivo* assays conducted with SWCNT (CNI, HiPco) and another type of SWCNT, leading the WHO work group to classify the entire SWCNT category to GHS Category 2. GreenScreen® criteria classify chemicals as a Moderate hazard for mutagenicity/genotoxicity when they are classified to GHS Category 2 (CPA 2018b). The confidence in the score is low due to the limited evidence and lack of Guideline DNA damage studies on various grades of SWCNTs.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - *In vitro*: Negative results for mutagenicity were obtained in a GLP-compliant bacterial reverse mutation assay conducted according to OECD Guideline 471. *Salmonella typhimurium* tester strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* tester strain WP_2 *uvr* A were treated with suspensions of Tuball™ SWCNT using both the Ames plate incorporation and pre-incubation methods at 10-5,000 µg/plate, with and without metabolic activation. No cytotoxicity or increase in the mutation frequency was observed in the presence or absence of metabolic activation. The vehicle and positive controls were valid (Klimisch 1, reliable without restriction).
  - *In vivo:* In a GLP-compliant *in vivo* mammalian alkaline comet assay conducted according to OECD Guideline 489, Crl: CD (SD) male rats (5/dose group) were administered suspensions of Tuball™ SWCNT in 1% Tween 80 as a single or repeated (intermittent) instillation for inducing acute or subacute inflammatory responses. In the single instillation

study, a dose of 1.0 mg/kg SWCNT was used for the high dosage group, which is expected to induce lung inflammation, and a dose of 0.2 mg/kg was used for the low dosage group, which is not expected to induce inflammation. In the repeated (intermittent) instillation study, a dosage of 0.2 or 0.04 mg/kg body weight once a week for 5 weeks was selected based on expected induction of sub-acute lung inflammation at the high dose but not at the low dose. Animals were sacrificed 3 or 24 hours after the single treatment, while in the repeated instillation group, rats were anesthetized and sacrificed 3 hours after the last treatment. The lungs were excised immediately after sacrifice; the left lobe was used for the histopathological examination, and the right lobe for the comet assay. There was no DNA damage in the lung cells of rats intratracheally instilled, even at doses that elicited both acute and subacute inflammatory responses. Accordingly, study authors concluded that SWCNTs have no potential for genotoxicity *in vivo (*Klimisch 1, reliable without restriction).

- OECD 2016, WHO 2017
    - *In vitro*: Negative results for mutagenicity were obtained in a bacterial reverse mutation assay conducted according to OECD Guideline 471 with Nikkiso SWCNT in the presence and absence of metabolic activation. *S. typhimurium* tester strains TA1535, TA1537, TA98 and TA100 and *E. coli* tester strain WP_2 *uvr* A were treated with test substance at the concentrations of 1.563, 3.125, 6.25, 12.5, 25, 50 and 100 µg/plate. No mutation induction was observed with or without metabolic activation in any of the tested concentration in any strain. Positive control showed expected levels of mutagenicity.
    - *In vitro*: Negative results for mutagenicity were obtained in a bacterial reverse mutation assay conducted according to the Japanese Guideline (Chemical Substances Control Law of Japan) with Super Growth SWCNT, in the presence and absence of metabolic activation. *S. typhimurium* TA 97, TA98, TA100, TA1535 and TA1537 and *E. coli* WP_2 *uvr*A/pkM101 were exposed to the test substance (suspended in 0.1% CMC-Na solution) at concentrations of 12.5, 25, 50, 100, 200 and 500 µg/plate. No mutation induction was observed with or without metabolic activation in any tested concentration in any the strain. Positive control showed expected levels of mutagenicity.
    - *In vitro*: Negative results for mutagenicity were obtained in a bacterial reverse mutation assay conducted with SWCNT (CNI, HiPco) using *S. typhimurium* YG1024 and YG1029 without metabolic activation at 0-240 µg/plate. No increases in mutation frequencies in either YG1024 or YG1029 were found at any concentrations of SWCNT.
    - *In vitro:* Negative results for clastogenicity were obtained in two chromosome aberration tests conducted according to OECD Guideline 473 with Nikkiso SWCNT and Super Growth SWCNT. Chinese hamster lung (CHL) cells were exposed to the test substance at concentrations of 6.25, 12.5, 25 and 50 µg/plate (Nikkiso SWCNT) or f 300, 500 or 1,000 µg/plate (Super Growth SWCNT), with and without metabolic activation. There was no evidence of induction of chromosomal aberrations, and positive controls produced the expected responses.
    - *In vitro:* Negative results for clastogenicity were obtained in an *in vitro* mammalian cell micronucleus test for SWCNT (CNI, HiPco) using CHL (V79) cells at a concentration of 0, 12, 24, 48 or 96 µg/cm^2 for 24 hours. No indications of chromosomal breakage and/or mitotic spindle damage were found.
    - *In vitro:* Positive results for DNA Damage were seen in a comet assay conducted with SWCNT (CNI, HiPco) using CHL (V79) cells at concentration of 0, 24, 48 or 96 µg/cm^2 for 3 or 24 h. A 3-hour SWCNT treatment led to DNA damage only at the highest SWCNT concentration. A 24-hour treatment led to DNA damage in a concentration-dependent manner. A 24-hour exposure to 48 µg/cm^2 of SWCNT significantly increased the level of

migrated DNA, tail length and olive tail moment by 2.25-, 1.76-, and 2.8-fold, respectively, while treatment with 96 μg/cm^2 SWCNT produced elevation in these parameters by 2.5-, 1.94-, and 3.4-fold, respectively.
    - *In vivo*: Nikkiso SWCNT and Super Growth were negative in two *in vivo* micronucleus tests conducted according to OECD Guideline 474 using Crlj:CD1(ICR) mice (5-6/dose) that received a single oral dose of the test substance via gavage at 5, 10 and 20 mg/kg/day (Nikkiso SWCNT) or at 60 or 200 mg/kg/day (Super Growth) and were sacrificed after 24 or 48 hours. There were no increases in micronuclei in the bone marrow.
    - *In vivo*: Nikkiso SWCNT was negative in a comet assay conducted in lung tissue taken from male rats exposed to the test substance at 0.2 or 1.0 mg/kg once or 0.04 or 0.2 mg/kg for 5 times (once/week) by intratracheal administration. There were no effects on %tail DNA.
    - *In vivo*: Positive results for DNA damage were seen in non-guideline *in vivo* assays conducted with SWCNT (CNI, HiPco) and another type of SWCNT. Increased K-ras mutations were found in lung tissue of mice following pharyngeal aspiration of the test substance at 0-20 μg/mouse. In addition, increased mitochondrial DNA damage was found in mice exposed to SWCNT (CNI, HiPco) at 10 and 40 μg/mouse by intrapharyngeal instillation and increased oxidative DNA damage was found in liver and lung tissue from Fisher rats exposed to another type of SWCNT by gavage at 0.064 or 0.64 mg/kg.
    - Based on the positive results seen for DNA damage with CNI, HiPco SWCNT (the induction of K-ras mutation in lung after inhalation in a non-guideline study in mice, and the evidence of genotoxicity in the *in vitro* Comet assay), the WHO working group classified the entire SWNCTs category as GHS Category 2 for germ cell mutagenicity with low confidence.
- Nordic Chemical Group 2019
    - The Nordic Chemical Group panel performed a GHS classification for the genotoxicity endpoint of SWCNTs and only the data on Nikkiso SWCNT and Super Growth SWCNT as well as data on CNI, HiPco SWCNT were considered as these types of SWCNT have been subject to the most thorough testing *in vitro* and *in vivo*. The negative results from testing of these types of SWCNT in bacteria were discounted as these tests are considered not relevant to nanomaterials since the nanomaterials may not be able to cross the bacterial wall. Based on the available data on Nikkiso SWCNT and Super Growth SWCNT, no classification for germ cell mutagenicity is warranted. Both substances were tested in an *in vitro* chromosomal aberration in mammalian cells (OECD Guideline 473), an i*n vivo* micronucleus testing in mice using oral exposure (OECD Guideline 474). Nikkiso SWCNT was further tested in a Comet assay in lung tissue from rats exposed by intratracheal administration. All these tests resulted in negative outcome, indicating lack of genotoxic potential. However, it is not known whether the negative result in the OECD Guideline 474 study is due to lack of distribution of the SWCNT to the bone marrow of the animals. CNI, HiPco SWCNT has been tested *in vitro* for micronucleus formation in CHL cells with negative outcome and in a Comet assay using CHL cells with positive outcome. It was also positive for mitochondrial DNA damage, increased K-ras mutation and induced mitotic spindle disruption. Accordingly, authors stated that a classification as Mutagen Category 2 may be warranted for this type of SWCNT. In addition, oxidative DNA damage in liver and lung tissue from rats orally exposed to another type of SWCNT, support concern for a possible genotoxic potential of SWCNTs. Based on this, the Nordic Chemical Group assigned a GHS Category 2 classification to the entire SWCNTs category and concluded that further *in vivo* testing on site-of contact tissues is required for various grades of SWCNTs to reach a conclusion on the mutagenicity potential of such materials.

Reproductive Toxicity (R) Score (H, M, or L): *L*
SWCNTs were assigned a score of Low for reproductive toxicity based on the lack of reproductive toxicity observed in a reproduction/developmental toxicity screening test in rats performed with Tuball™ SWCNT. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate data are available and negative and when they are not classified under GHS (CPA 2018b). The confidence in the score is low as it is based on data from a reproduction toxicity screening test that may not have examined all relevant endpoints.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - *Oral*: In a GLP-compliant combined repeated dose toxicity study with reproduction/developmental toxicity screening conducted according to OECD Guideline 421, male and female CD / Crl:CD rats (10/sex/dose) were administered Tuball™ SWCNT in the diet daily at doses of 0, 100, 300 or 1,000 mg/kg/day. Males were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and post-mating phase (11 - 14 days). Females were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and gestation and lactation phases (36 days). The parental animals were evaluated for clinical signs of toxicity, body weight, food consumption, estrus cyclicity, sperm parameters, histopathology of the female and male reproductive organs (testes and epididymis and ovarian and uterine content), and reproductive indices (fertility index, gestation index and viability index). Offspring were evaluated for survival, mean litter size, sex ratio, body weight, ano-genital distance, nipple retention (male pups), and external and internal abnormalities. There were no treatment related effects on any of the reproductive parameters measured in the treated male or female rats of this study. Based on this a NOAEL of 1,000 mg/kg/day was established for reproductive toxicity (Klimisch 1, reliable without restriction).

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *L*
SWCNTs were assigned a score of Low for developmental toxicity based on the absence of adverse developmental effects in a reproduction/developmental toxicity screening test in rats performed with Tuball™ SWCNT. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is low as it is based on data from a reproduction toxicity screening test that may not have examined all relevant endpoints.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - *Oral*: In the previously described GLP-compliant combined repeated dose toxicity study with reproduction/developmental toxicity screening conducted according to OECD Guideline 421, male and female CD / Crl:CD rats (10/sex/dose) were administered Tuball™ SWCNT in the diet daily at doses of 0, 100, 300 or 1,000 mg/kg/day. Males were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and post-mating phase (11 - 14 days). Females were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and gestation and lactation phases (36 days). Offspring were evaluated for survival, mean litter size, sex ratio, body weight, ano-genital distance, nipple retention (male pups), and external and internal abnormalities. There were

no embryotoxic or teratogenic effects observed with treatment. The study authors identified the developmental toxicity NOAEL as 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction).

- WHO 2011, OECD 2016
    - In developmental toxicity and teratogenicity studies, Pregnant CD-1 mice were intravenously injected with SWCNT, oxidized-SWCNT and ultra oxidized-SWCNT at 0 - 30 μg/animal on day 5.5 of gestation. In dams, no adverse effects were observed except in placenta. A high percentage of early miscarriages or fetal malformations was observed in the oxidized SWCNT group, while lower percentages were observed in SWCNT group at 0.1 μg/animal and higher. The LOAEL of reproductive and developmental toxicity of SWCNTs was considered to be 0.1 μg/animal. The WHO panel stated that although these data suggest some form of developmental toxicity, the experiment was not conducted based on test guidelines and the exposure route was not unconventional for SWCNTs, thus it is difficult to categorize reproductive and developmental toxicity. Based on this, no developmental toxicity hazard classification was assigned for SWCNTs in the WHO report.

Endocrine Activity (E) Score (H, M, or L): DG
SWCNTs were assigned a score of Data Gap for endocrine activity based on lack of data for this endpoint.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- No data were identified.

Group II and II* Human Health Effects (Group II and II* Human)
Note: Group II and Group II* endpoints are distinguished in the v 1.4 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. See GreenScreen® Guidance v1.4, Annex 2 for more details.

Acute Mammalian Toxicity (AT) (Group II) Score (vH, H, M, or L): *L*
SWCNTs were assigned a score of Low for acute toxicity based on its expected lack of bioavailability supported by limited data on one type of SWCNT (Nikkiso) with oral LD_{50} > 50 mg/kg (the maximum achievable concentration). GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral and dermal LD_{50} values are > 2,000 mg/kg, and inhalation LC_{50} values are > 5 mg/L/4h (dust) and/or when they are not classified per GHS (CPA 2018b). The confidence in the score is low as it is based on expert judgment and due to lack of guidance for testing voluminous nanomaterials for GHS classification purposes as testing SWCNT with a high specific volume (i.e., volume per unit of mass) is only achievable at low dose levels far below the GHS threshold value of 2,000 mg/kg/day for oral and dermal acute toxicity and 5 mg/L/4h for acute inhalation toxicity (dust). Further, only tests on one type of SWCNT are available.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
    - It is technically not feasible to conduct acute oral and inhalation toxicity studies on Tuball™ SWCNT as the test item was found to be impossible to formulate satisfactorily in a suitable vehicle for oral or inhalation dosing. It is the opinion of the registrant manufacturer (Envigo Research Limited) that the bioavailability of Tuball™ SWCNT is negligible, due to its inert

nature, and it is reasonable to assume that the test item presents no significant acute toxicity risk under the conditions of the test.

- OECD 2016, WHO 2017
  - *Oral*: LD_{50} (Crl:CD(SD) female rats) > 50 mg/kg for Nikkiso SWCNT (OECD Guideline 423). The maximum dose of 2,000 mg/kg required by the guideline could not be used due to very high specific volume of SWCNT.
  - *Oral:* In two *in vivo* micronucleus studies conducted according to OECD Guideline 474, Male Crlj:CD1(ICR) mice (5 or6 /dose group) were administered Nikkiso SWCNT (suspended in the water and was diluted with 0.3% CMC-Na solution) and Super Growth SWCNT (suspended in PBS with 1% Tween 80) by gavage at doses of 5, 10 or 20 mg/kg/day or 60, 200 mg/kg/day, respectively, two times in the interval of 24 hours. No death or indicative of abnormality is observed in both studies.
  - *Inhalation:* In an acute inhalation toxicity study, female C57BL mice were exposed to non-purified SWCNT (iron content of 17.7% by weight) via whole body inhalation at concentration of 5 mg/m^3, 5 h/day for 4 days. No mortality was observed. The study reported that 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 in the lungs of mice. This was probably due to the higher content of iron.
  - Based on the results from the above studies with Nikkiso and Super Growth SWCNTs, no acute toxicity hazard classification was assigned for SWCNTs in the WHO report with the level of evidence being moderate to strong for oral route of exposure and moderate to weak for inhalation.
- Nordic Chemical Group 2019
  - Due to the physical chemical properties of SWCNT as an insoluble substance, dermal absorption is considered very low/negligible, and thus a potential for acute dermal toxicity seems unlikely. The oral and dermal acute toxicity test methods seem only to be applicable for SWCNT at low dose levels; a higher dose level was impracticable because of very high specific volume of SWCNT. Accordingly, GHS classification cannot be concluded due to lack of guidance for testing voluminous nanomaterials. The Nordic Chemical Group concluded that for the Nikkiso SWCNT "no classification" for acute oral toxicity is warranted based on insufficient data.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) (Group II) Score (vH, H, M, or L): DG

SWCNTs were assigned a score of Data Gap for systemic toxicity (single dose) based on lack of sufficient data for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- WHO 2017
  - No acute toxicity studies using normal exposure routes were identified. Other studies using intratracheal instillation, pharyngeal aspiration and intraperitoneal injection of SWCNTs were conducted *in vivo* in experimental animals with various doses and observation periods. The results of the intratracheal instillation and pharyngeal aspiration studies showed some degree of lung damage with elevation of various biomarkers. However, these studies were not conducted using standard exposure routes and according to test guidelines, so it was difficult to categorize the respective SWCNTs under GHS.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*) Score (H, M, or L): *H*

SWCNTs were assigned a score of High for systemic toxicity (repeated dose) based on WHO's classification of the entire SWCNT group to GHS Catgeory1 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 (CPA 2018b). The confidence in the score is low as the available evidence for classification were considered weak by the WHO.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS – Australia - H373 - May cause damage to organs through prolonged or repeated exposure
- ECHA 2021a
  - *Oral*: In the previously described GLP-compliant combined repeated dose toxicity study with reproduction/developmental toxicity screening conducted according to OECD Guideline 421, male and female CD / Crl:CD rats (10/sex/dose) were administered Tuball™ SWCNT in the diet daily at doses of 0, 100, 300 or 1,000 mg/kg/day. Males were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and post-mating phase (11 - 14 days). Females were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and gestation and lactation phases (36 days). The parental animals were evaluated for clinical signs of toxicity, body weight, food consumption, hematology, clinical chemistry, organ weight, gross pathology, and histopathology. There were no treatment related effects on any of these parameters. Authors assigned a NOAEL of 1,000 mg/kg/day for systemic toxicity, the highest dose tested (Klimisch 1, reliable without restriction).
- OECD 2016, WHO 2017
  - *Oral*: In a repeated dose toxicity study conducted according to OECD Guideline 407, male and Female Crl:CD rats (5 or 10 /sex/dose) were administered Nikkiso SWCNT (suspended in 5% guam acacia) by gavage at doses of 0, 0.125, 1.25 or 12.5 mg/kg/day for 28 days with a 14-day recovery period (0 and 12.5 mg/kg/day groups). The maximum dose of 1,000 mg/kg required by the guideline was impracticable because of very high specific volume of SWCNT. No treatment related changes of body weight, behavioral and blood biochemical parameters were observed. A few minor changes with statistical significance in white blood cells composition, organ weights and urine volume were detected, although no relevant pathological changes were observed. Based on this, authors assigned a NOAEL of 12.5 mg/kg/day for systemic toxicity, the highest dose tested.
  - *Inhalation*: In two 28-day repeated dose toxicity studies conducted according to OECD Guideline 412, Wistar rats were exposed to Nikkiso SWCNT and Super Growth SWCNT particles 6 hours/day, 5 days/week for 4 weeks at exposure levels of 0; 0.08 and 0.40 mg/m^3 or 0 mg/m^3, 0.03 and 0.13 mg/m^3, respectively. The particle number concentrations in the two groups for each test substance were 5.0 ± 0.7 x 104 and 6.6 ±2.1 x 104 particles/cm^3, respectively. In both studies, inflammation and fibrotic response were examined 3 days, 1 month and 3 months after exposure. Treatment with Nikkiso SWCNT caused increased neutrophil cells in blood at 3 months after administration in the high concentration group. No adverse pulmonary effects or signs of neutrophil inflammation were noted in the study with Super Growth SWCNT. A no adverse effect concentration (NOAEC) of 0.13 mg/m^3 was suggested for Super Growth SWCNT. Based on the results from the study with Nikkiso SWCNT, the WHO classified the entire group of SWNCTs to GHS Category 1 for specific

organ toxicity upon repeated exposure (inhalation) with the evidence considered as weak. *The LOAEC of 0.4 mg/m^3 (equivalent to 0.28 mg/m^3/6h/day[12]) is below the duration-adjusted GHS guideline value for Category 1 of 60 mg/m^3/6h/day[13] (dust) for 28-day studies.*

- OECD 2016
  - *Intratracheal*: Super Growth SWCNT (suspended in PBS with 1% Tween 80) was administrated to male Crl:CD(SD) rats by intratracheal instillation for 5 times (once a week) at 0, 0.04 or 0.2 mg/kg. BALF was examined at 1, 4, or 13 weeks after last instillation. Body weight and food consumption were not affected. Increases in white blood cells, eosinophils, proteins, LDH and IL-1β were measured up to 13 weeks of the observation period at 0.04 and 0.2 mg/kg. At 0.2 mg/kg, increases in lung weight were measured up to 13 weeks. Histopathological examination revealed aggregation of macrophages at 0.04 and 0.2 mg/kg.
  - *Pharyngeal aspiration*: In one non-guideline study, ApoE-/- mice were repeatedly dosed by pharyngeal aspiration to SWCNT (CNI, HiPco) at 20 µg/mouse once every second week for 8 weeks. Histopathological examination showed a significant increase in the plaque formation in the aorta.
- Nordic Chemical Group 2019
  - *Inhalation:* In a short term non- guideline inhalation toxicity study, mice (number and sex not specified) were exposed to SWCNT (CNI, HiPco) at a dose of 5.52 ±1.37 mg/m^3, 5 hours daily for 4 days (type of exposure; nose or whole body is not specified). Treatment caused a statistically significant increase in LDH accumulation in BAL fluid of mice that inhaled SWCNT (118%, 80%, and 71% compared to the control groups) throughout the recovery period (1, 7, and 28 days post-exposure). Histopathological examination of four mice at 28 days post-exposure revealed bronchiolar epithelial cell hypertrophy with one mouse having both hypertrophy and hyperplasia, one mouse having peribronchiolar bronchiolization accompanying bronchiolar epithelial cell hypertrophy, and two mice having bronchiolar epithelial cell hypertrophy without other bronchiolar alterations. Further, foci of granulomatous inflammation were noted with fibrosis. Accordingly, authors concluded that inhalation of SWCNT resulted in an inflammatory response, oxidative stress, collagen deposition, and fibrosis in the lung 28 days post-exposure. However, authors also stated that the SWCNT used was non-purified and as produced, having a diameter of 0.8-1.2 nm, a length of 100–1,000 nm and a content of 82% elemental carbon, 17.7% iron, 0.16% copper, 0.049% chromium, and 0.046% nickel. It is important to note the content of transition metals (especially the high content iron) as these transition metals can act as prooxidants. Thereby a combination of inflammatory response with catalytic metal-containing carbon nanotubes would synergistically enhance damage to cells and tissues.
  - The Nordic Chemical Group panel performed a GHS classification for specific target organ toxicity (repeated dose) (STOT RE) endpoint on SWCNTs. The panel classified SWCNTs to GHS Category 1 for STOT following repeated inhalation exposure with the lung identified as the target organ. The classification was based on the results from the 4-day study with SWCNT (CNI, HiPco) supported by data from studies using single dose exposure to SWCNT by intratracheal instillation or pharyngeal aspiration. The two 28-day inhalation toxicity studies that were conducted with Nikkiso SWCNT and Super Growth SWCNT were considered by the panel to be insufficient for GHS classification since no effects were seen in these studies with the highest concentrations used being considerably lower than the GHS Guidance value for Category 2 classification of 600 mg/m^3 for a 28-day study. *ToxServices*

[12] Converting exposure period 5days/week to daily = 0.4 mg/m^3 x 5 / 7(days) = 0.28 mg/m^3/day
[13] 0.02 mg/L (20 mg/m^3) x 90 days /28 days = 60 mg/m^3

noted that the 28-day repeated inhalation toxicity study with Nikkiso SWCNT described in the Nordic Chemical Group document did not report the effect observed on neutrophils in blood.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): DG
SWCNTs were assigned a score of Data Gap for neurotoxicity (single dose) based on lack of sufficient data for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- No data were identified.

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): L
SWCNTs were assigned a score of Low for neurotoxicity (repeated dose) based on a lack of effects on neurological endpoints at doses up to 1,000 mg/kg/day in a 90-day repeated dose toxicity study performed with Tuball™ SWCNT. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when they are not classified under GHS based on a lack of effects on neurological endpoints below the Guidance value of 100 mg/kg/day for a 90-day oral study (CPA 2018b). The confidence in the score is high as it is based on measured data of high quality for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - *Oral*: In the previously described GLP-compliant combined repeated dose toxicity study with reproduction/developmental toxicity screening conducted according to OECD Guideline 421, male and female CD / Crl:CD rats (10/sex/dose) were administered Tuball™ SWCNT in the diet at daily doses of 0, 100, 300 or 1,000 mg/kg/day. Males were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and post-mating phase (11 - 14 days). Females were exposed to the test substance during pre-mating phase (14 days), mating phase (2 - 5 days) and gestation and lactation phases (36 days). Animals were evaluated for motor activity (MA), sensory reactivity and grip length. There were no changes in any of the parameters tested. A neurotoxicity NOAEL of 1,000 mg/kg/day was established based on the lack of effects at the highest dose tested (Klimisch 1, reliable without restriction). *The dose of 1,000 mg/kg/day is above the GHS Category 2 cut-off value of 100 mg/kg/day for a 90-day study. Therefore, the test substance is not classified per GHS.*

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): L
SWCNTs were assigned a score of Low for skin sensitization based on negative results in skin sensitization studies performed with three types of SWCNTs (Tuball®, Nikkiso, and Super Growth). GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative, and when they are not classified per GHS (CPA 2018b). The confidence in the score is high as it is based on measured data of good quality for the target chemicals.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a

    - Tuball™ SWCNT was not sensitizing in a GLP-compliant Buehler skin sensitization study conducted according to OECD Guideline 406. Female Hartley guinea pigs (20/dose) were intradermally induced with 20% of the test substance in Vaseline as 3 topical applications under occlusive dressing for 6 hours with a 13-day rest phase. The animals were then challenged with 20%. No positive reactions were observed. The authors concluded that Tuball™ SWCNT is not sensitizing under the conditions of the assay (Klimisch 1, reliable without restriction).
- OECD 2016, WHO 2017
    - Both Nikkiso and Super Growth SWCNTs were non-sensitizing to the skin of male guinea pigs (n = 20) when tested according to OECD Guideline 406. No clinical signs or changes in body weight gain were observed in any group. No erythema or edema was observed after the challenge with the test substances. Accordingly, no skin sensitization hazard classification was assigned for SWCNTs in the WHO report with the evidence being considered as strong.

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): *L*
SWCNTs were assigned a score of Low for respiratory sensitization based on the negative skin sensitization data and according to ECHA's recommended strategy on evaluation of respiratory sensitization. GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low as this evaluation does not include non-immunologic mechanisms of respiratory sensitization, and no specific data are available for respiratory sensitization.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- WHO 2017
    - SWCNTs are not respiratory sensitizers based on negative results in skin sensitization studies performed with Nikkiso and Super Growth SWCNTs. Based on this, no respiratory sensitization hazard classification was assigned for SWCNTs in the WHO report with the evidence being considered as strong.
- Based on the weight of evidence and guidance from ECHA regarding assessment of respiratory sensitization potential, a score of Low was assigned. The guidance from ECHA states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017). As SWCNTs were not sensitizing to the skin (see skin sensitization section above), and a literature search did not find any human evidence of respiratory sensitization by SWCNTs, they are not expected to be respiratory sensitizers.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): *L*
SWCNTs were assigned a score of Low for skin irritation/corrosivity based on negative results in dermal irritation studies performed with three types of SWCNTs (Tuball®, Nikkiso, and Super Growth). GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate data are available and negative, and they are not GHS classified (CPA 2018b). The confidence in the score is low as the *in vivo* studies applied very low doses of the test substances compared to the recommended dose per the OECD 404 Guideline study (0.5 g for solid substances) and the *in vitro*

OECD 439 test cannot discriminate mild skin irritants (Category 3) from substances not classified per GHS (UN 2019).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - Tuball™ SWCNT was considered to be non-irritating (GHS Category 2) to the skin when tested in a GLP-compliant *in vitro* skin irritation test conducted according to OECD Guideline 439 using reconstructed human Epidermis (RHE) Skin Model after treatment periods of 3 and 60 minutes and a 72-hour post-exposure incubation period. The relative mean viabilities of the test item were 97.8% after 3 minutes and 97.9% after 60 minutes, which are greater than 50% and indicate the substance is not irritating to the skin (Klimisch 1, reliable without restriction).
  - Tuball™ SWCNT was considered to be non-irritating (GHS Category 2) to the skin when tested in a GLP-compliant *in vitro* skin irritation test conducted according to OECD Guideline 439 using reconstructed EpiDerm Human Skin Model after treatment periods of 15 minutes and a 42-hours post-exposure incubation period. The relative mean viability of the test item was 101.3% after the 15-minutes exposure period, which is greater than 50% and indicate the substance is not irritating to the skin (Klimisch 1, reliable without restriction).
- OECD 2016, WHO 2017
  - In two skin irritation studies conducted according to OECD Guideline 404, Nikkiso SWCNTs were not irritating to the rabbit skin when applied to three male Kbl:NZW rabbits as 0.5 g solution of 1 wt% in olive oil, 0.5 ml of 0.3 wt% in silicon oil, or powder of 0.02 g sopped with silicon oil. No clinical signs or changes in body weight gain were observed in any groups treated with SWCNTs. No dermal responses, including erythema/eschar or edema, were found in rabbits. The studies were considered in the WHO report to be of high quality.
  - Super Growth SWCNT was not irritating to the skin or rabbits when tested in a skin irritation study conducted according to OECD Guideline 404. Super Growth SWCNT solution (0.5g of 1 wt% in olive oil; maximum concentration that could be prepared) was applied to three male Kbl:NZW rabbits. No clinical signs or changes in body weight gain were observed in any groups treated with SWCNTs. No dermal responses, including erythema, eschar and edema, were found in rabbits. The study was considered in the WHO report to be of high quality.
  - Based on the results from the above studies with Nikkiso and Super Growth SWCNTs, no skin irritation hazard classification was assigned for SWCNTs in the WHO report with the evidence being considered as strong.

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): *H*

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. GreenScreen® criteria classify chemicals as a High hazard for eye irritation/corrosivity when they are classified to GHS Category 2A (CPA 2018b). The 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. The test cannot discriminate eye irritants (Category 2) from substances causing serious eye damage (Category 1) (ECHA 2017). In addition, no GHS classification criteria have been adopted yet for test results from *in vitro* studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a, Nordic Chemical Group 2019
  - A GLP-compliant *in vitro* ocular irritation test conducted according to OECD Guideline 492 was performed with reconstructed human cornea-like epithelium (RhCE) exposed to 50 mg undiluted Tuball™ SWCNT for six hours. The cells were then maintained for an additional 18 hours without treatment. The final cell viability was 26.5%, indicating relevant irritating effects (threshold for irritancy: ≤ 60%). Based on this, authors of REACH dossier and the Nordic Expert Group concluded that Tuball™ SWCNT is irritating to the eye and is classified to GHS Category 2 per the EU-GHS (CLP) (Klimisch 1, reliable without restriction). *This is equivalent to GHS Category 2A per GHS as Category 2B was not adopted by the EU-GHS.*
- OECD 2016, WHO 2017
  - In two ocular irritation studies conducted according to OECD Guideline 405, Nikkiso and Super Growth SWCNTs were non-irritating to the rabbit eye. Three male Kbl:NZW rabbits received an instillation of 0.1 mL test sample solution containing 0.1 wt% of SWCNTs in olive oil (maximum achievable concentration of test material). No clinical signs or changes in body weight gain were observed. Ocular responses, such as corneal opacity, conjunctival redness, abnormality of the iris, and chemosis, were not detected in rabbits at any observation period.
  - Based on the results from the above studies with Nikkiso and Super Growth SWCNTs, no eye irritation hazard classification was assigned for SWCNTs in the WHO report with the evidence being considered as strong. However, the WHO panel stated that caution should be exercised for irritation because MWCNTs, which have similar material properties, did show GHS Category 2 eye irritation/corrosion with strong evidence.
- Nordic Chemical Group 2019
  - The Nordic Expert Group considered the above two studies not applicable for GHS classification purpose since the dose applied (0.1 mg of SWCNT) is significantly below the recommended GHS dose for such studies (0.1 mL or up to 100 mg of the test substance should be used when testing solids and particulate substances). The panel also stated that guidance may be needed on the relevance of *in vivo* testing for eye damage/ irritation of insoluble nanomaterials with high specific volume.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): L

SWCNTs were assigned a score of Low for acute aquatic toxicity based on measured LC_{50}/EC_{50} values of > 10 mg/L in fish, daphnia, and algae for two types of SWCNT (Nikkiso and Super Growth) indicating lack of toxicity at saturation. GreenScreen® criteria classify chemicals as a Low hazard for acute aquatic toxicity when acute aquatic toxicity values are greater than 100 mg/L and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on measured data of high quality in the three trophic levels for the target chemicals.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2016

- 96-hour LC_{50} (*Oryzias latipes*, fish) > 10 mg/L for both Nikkiso and Super Growth SWCNTs. No mortality was observed at the concentrations of 10 mg/L (GLP-compliant, OECD Guideline 203).
- 4 to 96-hour post fertilization LOEC (*Danio rerio*, zebra fish) = 120 mg/L for SWCNTs from Sigma Aldrich (11 nm average diameter, 0.5 – 100 um average length, purity of 90 atom percent, covered with negatively charged carboxylic acid grouped at the defect sites on their sidewalls, and contain cobalt and nickel as impurities). Delayed hatching was found at concentrations of 120 mg/L, but this was attributed to the cobalt and nickel catalyst contaminants present in the test substance.
- 48-hour EC_{50} (*Daphnia. magna*, invertebrate) > 10 mg/L for both Nikkiso and Super Growth SWCNTs (OECD Guideline 202).
- 48-hour EC_{50} (*D. magna*, invertebrate) = 1.306 mg/L (immobilization) for SWCNTs from Shenzhen Nanotech (diameter < 2 nm, length 5-15 um, purity: SWCNT > 60, CNT > 90%) (OECD modified Guideline 202).
- 72-hour EC_{50} (*Pseudokirchneriella subcapitata*, green algae) for growth rate and biomass > 10 mg/L for both Nikkiso and Super Growth SWCNTs (OECD Guideline 201).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *H*
SWCNTs were assigned a score of High for chronic aquatic toxicity based on measured LOEC values of 1 mg/L in daphnia and algae for two types of SWCNT (Nikkiso and Super Growth). 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 (CPA 2018b). The confidence in the score is low due to lack of study details of the observed effects.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2016
  - 14-day NOEC (*O. latipes*, fish) > 10 mg/L for both Nikkiso and Super Growth SWCNTs. No mortality was observed at the concentrations of 10 mg/L (OECD Guideline 204).
  - 21-day NOEC (*D. magna*, invertebrate) = 0.32 mg/L (reproduction) for Nikkiso SWCNT (LOEC = 1 mg/L) and 0.3 mg/L for Super Growth SWCNT, the highest dose tested (OECD Guideline 211).
  - 72-hour NOEC (*P. subcapitata*, green algae) for growth rate = 0.32 mg/L for both Nikkiso and Super Growth SWCNTs (LOEC = 1.0 mg/L, OECD Guideline 201).
  - In a lifetime test using *Amphiascus tenuiremis* on SWCNT using the bench-scale arc-discharge method, NOECs are 1.6 mg/L for as prepared SWCNTs and 10 mg/L for purified SWCNTs.

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *vH*
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 with two types of SWCNT (Nikkiso and Super Growth). 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 (CPA 2018b). The confidence in the score is reduced as no experimental data are available that last more than 28 days.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2016
  - In two GLP-compliant biodegradation tests conducted according to OECD Guideline 301F (Manometric respirometry method), activated sludge was exposed to Nikkiso and Super Growth SWCNTs at concentration of 100 mg/L for 28 days. Biodegradation by BOD after a 28-day cultivation period was 0 %.
  - In another four biodegradation tests conducted according to OECD Guidelines 301C (Modified MITI method (I)) and 302C (Inherent Biodegradability: Modified MITI method (II)), no degradation was achieved with Nikkiso and Super Growth SWCNTs by the end of the 28-day exposure period.

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL
SWCNTs were assigned a score of Very Low for bioaccumulation based on several SWCNTs having measured BAF values of less than 1. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when BCF/BAF values are ≤ 100 (CPA 2018b). The confidence in the score is high as it is based on measure data for the target chemicals.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2016
  - The bioaccumulation potential of several SWCNTs in marine benthic organisms was evaluated at the base of the food chain. These SWCNTs included SG65, SG76, and CG100 produced by SouthWest NanoTechnologies using CoMoCAT method and 14C-radiolabeled SWCNT produced by Research Triangle Institute using the arc discharge method. No significant mortalities were seen in the *amphipod Ampelisca abdita* or the *mysid Americamysis bahia* at measured food- and sediment-borne concentrations of SWCNTs of up to 100 ppm (highest concentration tested) over a seven-day exposure period using static conditions. Similarly, no significant mortality was observed in *Leptocheirus plumulosus* exposed to sediment and/or food (*Isochrysis galbana*, an alga) spiked with 14C-radiolabeled SWCNTs in 28-day static renewal tests. In other treatments (10 µg/g sediment; 10 µg/g dry weight algae), bioaccumulation was limited with reported BAFs in amphipod/sediment and algae less than 1, and these decreased following depuration by approximately one order of magnitude. Accordingly, authors concluded that SWCNTs are not bioaccumulative in benthic organisms which take up the SWCNTs through ingestion and then rapidly eliminate them during depuration (radioactivity measured in fecal pellets, where SWCNTs added to sediment or food was significant relative to controls).
  - No bioaccumulation of SWCNT (another type supplied by SWeNT) was observed in *A. abdita* and *A. bahia*. *Cyclotella sp* (algae) and *Artemia salina* (brine shrimp) were spiked at 1 µg/g of SWCNT, respectively, as prey for *A. abdita* and *A. bahia* and trophic transfer was measured using novel near infrared fluorescence (NIRF) spectroscopic method.

<u>Physical Hazards (Physical)</u>

Reactivity (Rx) Score (vH, H, M, or L): *L*
SWCNTs were assigned a score of Low for reactivity based on Tuball™ SWCNT not being considered an explosive or oxidizing solid in its SDS supported by lack of structural alerts for explosivity.

GreenScreen® criteria classify chemicals as a Low hazard for reactivity when available data indicate that the chemical does not warrant GHS classification for any of the reactivity sub-endpoints and the chemical is not present on authoritative or screening list (CPA 2018b). The confidence in the score is low due to lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021a
  - No measured data were identified. Therefore, screening procedures for explosivity were used by the authors of REACH dossier to estimate the reactivity property of Tuball™ SWCNT. These procedures are listed in the GHS (UN 2019).
  - Tuball™ SWCNT is not considered explosive or self-reactive due to lack of functional groups associated with explosive or self-reactive properties (See Appendix G).
  - Tuball™ SWCNT is not considered to have oxidizing properties as it does not contain any structural groups known to be correlated with a tendency to react exothermally with combustible materials.
- OCSiAl 2020
  - A safety data sheet for Tuball™ SWCNT states that it is not an explosive or oxidizing solid.

Flammability (F) Score (vH, H, M, or L): *L*

SWCNTs were assigned a score of Low for flammability based on Tuball™ SWCNT not being classified as a flammable solid in its SDS. GreenScreen® criteria classify chemicals as a Low hazard for flammability when adequate data are available and negative, and they are not GHS classified (CPA 2018b). The confidence in the score is low due to lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OCSiAl 2020
  - A safety data sheet for Tuball™ SWCNT states that it is not a flammable solid.

Use of New Approach Methodologies (NAMs)[14] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* tests for genotoxicity, skin irritation and eye irritation. NAMs are non-animal alternative that can be used alone or in combination to provide information for safety assessment (Madden et al. 2020). At present, there is not a uniformly accepted framework on how to report and apply individual NAMs (U.S. EPA 2020b, OECD 2020). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by EFSA (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020):

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

As shown in Table 4, no Type I (input data) uncertainties on using SWCNTs' NAMs dataset (*in vitro* genotoxicity, skin and eye irritation tests) are identified. SWCNTs' Type II (extrapolation output) uncertainties include the limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, the non-applicability of the bacterial reverse mutation test to nanomaterials, the limitation of *in vitro* skin irritation test **(**RHE, OECD Guideline 439) to identify substances classified as mild skin irritant (GHS Category 3), and the limitation of the *in vitro* eye irritation test **(**RhCE test, OECD Guideline 492) to differentiate between Category 2 and Category 1, or between Category 2A and Category 2B. The type II errors can be alleviated by the use of genotoxicity test batteries *and in vivo* data for skin and eye irritation as there are no validated *in vitro* methods available for the direct identification of Category 2 eye irritants and Category 3 skin irritants.

Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Genotoxicity, Skin irritation, and Eye irritation**: No Type I uncertainty is identified on using the *in vitro* genotoxicity, skin, and eye irritation tests as they are considered relevant (appropriate for the evaluation of the corresponding hazards as recommended in the ECHA Guidance), reliable (they have Klimisch scoring of 2 or 1) and adequate (validated methods) (ECHA 2017).
Type II Uncertainty: Extrapolation Output	**Genotoxicity:** The *in vitro* bacterial mutagenicity testing is not recommended for nanomaterials as the nanomaterials may not be able to cross the bacterial wall (ECHA 2020). The *in vitro* chromosome aberration assay (OECD 473) does not measure aneuploidy and it only measures structural chromosomal

[14] NAMs refers to any non-animal technology, methodology, approach, or combination thereof that inform chemical hazard and risk assessments. NAMs include *in silico*/computational tools, *in vitro* biological profiling (e.g., cell cultures, 2,3-D organotypic culture systems, genomics/transcriptomics, organs on a chip), and frameworks (i.e., adverse outcome pathways (AOPs), defined approaches (DA), integrated approaches to testing and assessment (IATA).

	aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[15]. **Skin irritation**: The RHE test (OECD 439) is only used to identify irritating substances (GHS Category 2) and non-irritating (no category). It does not differentiate between these two classes or allow the classification as a mild skin irritant (GHS Category 3) (ECHA 2017). **Eye irritation**: The RhCE test (OECD 492) cannot differentiate between Category 2 and Category 1, or between Category 2A and Category 2B. There is no single *in vitro* method that can replace an *in vivo* eye irritation test[16]. Therefore, this method is not recommended for identifying eye irritants (Category 2) or substances causing serious eye damage (Category 1) (ECHA 2017).	
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	N	
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/ *in vitro* chromosome aberration assay/ *in vitro* comet assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	N	
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	N	
Respiratory sensitization	N	
Skin irritation	Y	*In vitro* test: OECD Guideline 439 (reconstructed human epidermis (RHE) test method)
Eye irritation	Y	*In vitro* test: OECD Guideline 492 (reconstructed human cornea-like epithelium (RhCE) test method)
Acute aquatic toxicity	N	

[15] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352
[16] https://www.oecd.org/env/ehs/testing/E492_2017.pdf

Chronic aquatic toxicity	N	
Persistence	N	
Bioaccumulation	N	

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APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for SWCNTs (CAS #308068-56-6)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicit	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details									S	R *	S	R *	*	*								
Inorganic Chemical?	**Chemical Name**	**CAS#**	**C**	**M**	**R**	**D**	**E**	**AT**	**STs**	**STr**	**Ns**	**Nr**	**SNS***	**SNR***	**IrS**	**IrE**	**AA**	**CA**	**P**	**B**	**Rx**	**F**
Yes	**SWCNTs**	**308068-56-6**	DG	*M*	*L*	*L*	DG	*L*		*H*		**L**	**L**	*L*	*L*	*H*	**L**	*H*	*vH*	**vL**	*L*	*L*

Table 3: Hazard Summary Table

Benchmark	a	b	c	d	e	f	
1	No	Yes	No	No			
2	STOP						
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
SWCNTs	1

Note: Chemical has not undergone a data gap assessment.
Not a Final GreenScreen™ Score

Table 6

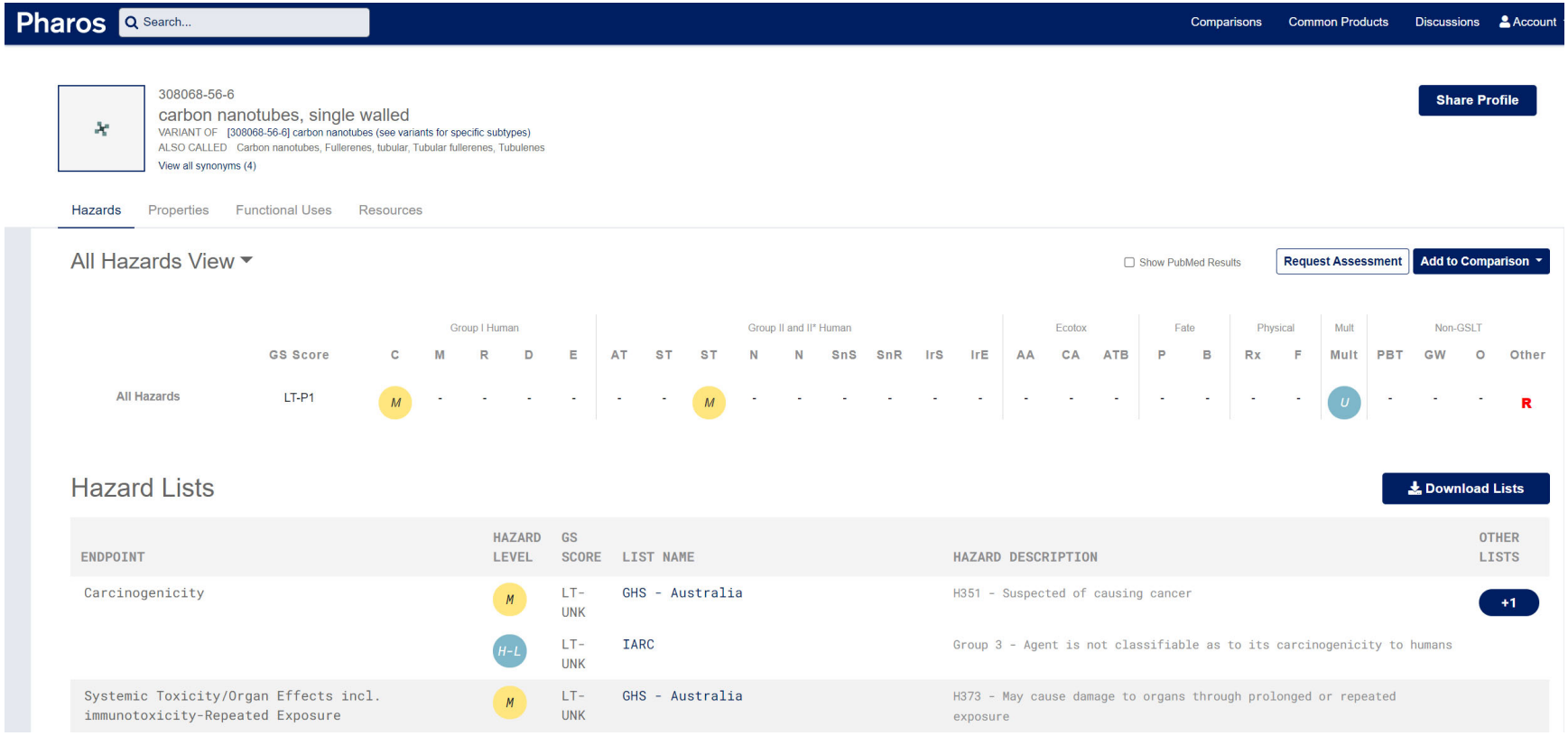
Chemical Name	Final GreenScreen® Benchmark Score
SWCNTs	1

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												1
2												
3												
4												

APPENDIX C: Pharos Output for SWCNTs (CAS #308068-56-6)



Carcinogenicity, Mutagenicity/Genotoxicity Reproductive Toxicity and/or Developmental Toxicity	U	LT-P1	ChemSec - SIN List	CMR - Carcinogen, Mutagen &/or Reproductive Toxicant

Restricted Substance Lists (2)

- CA SCP - Candidate Chemicals: Candidate Chemical List
- MDH - Chemicals of High Concern and Priority Chemicals: Chemicals of High Concern

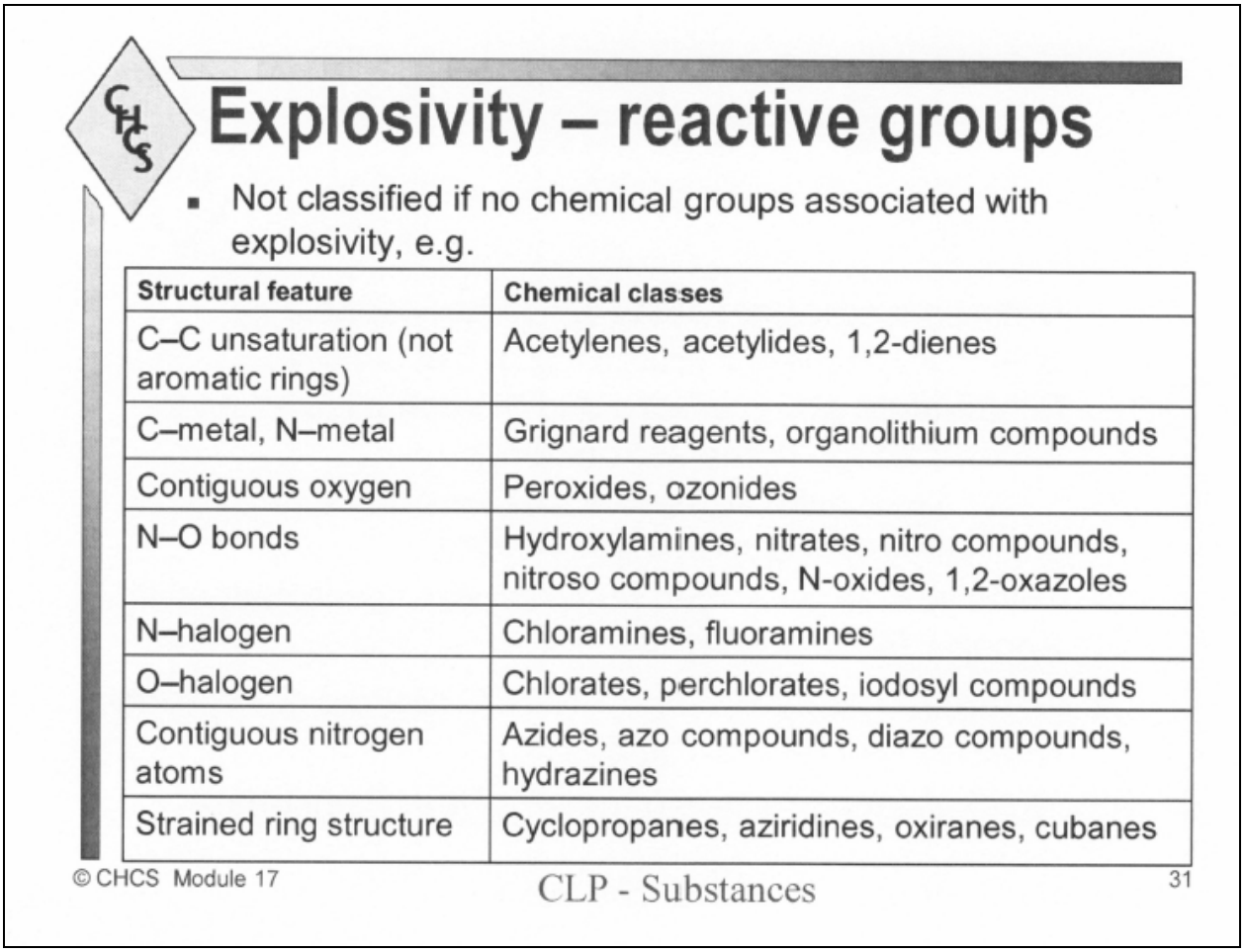
Discussions

No discussions have been posted yet.

Ask a question about this chemical in the forums >

APPENDIX D: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



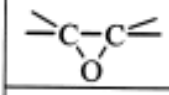
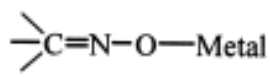
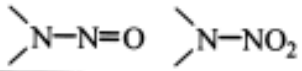
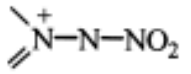
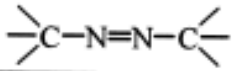
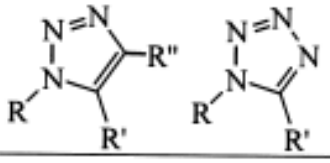
Explosivity – reactive groups

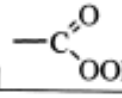
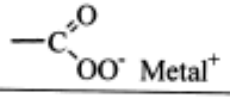
- Not classified if no chemical groups associated with explosivity, e.g.

Structural feature	Chemical classes
C–C unsaturation (not aromatic rings)	Acetylenes, acetylides, 1,2-dienes
C–metal, N–metal	Grignard reagents, organolithium compounds
Contiguous oxygen	Peroxides, ozonides
N–O bonds	Hydroxylamines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles
N–halogen	Chloramines, fluoramines
O–halogen	Chlorates, perchlorates, iodosyl compounds
Contiguous nitrogen atoms	Azides, azo compounds, diazo compounds, hydrazines
Strained ring structure	Cyclopropanes, aziridines, oxiranes, cubanes

Explosivity – Full List

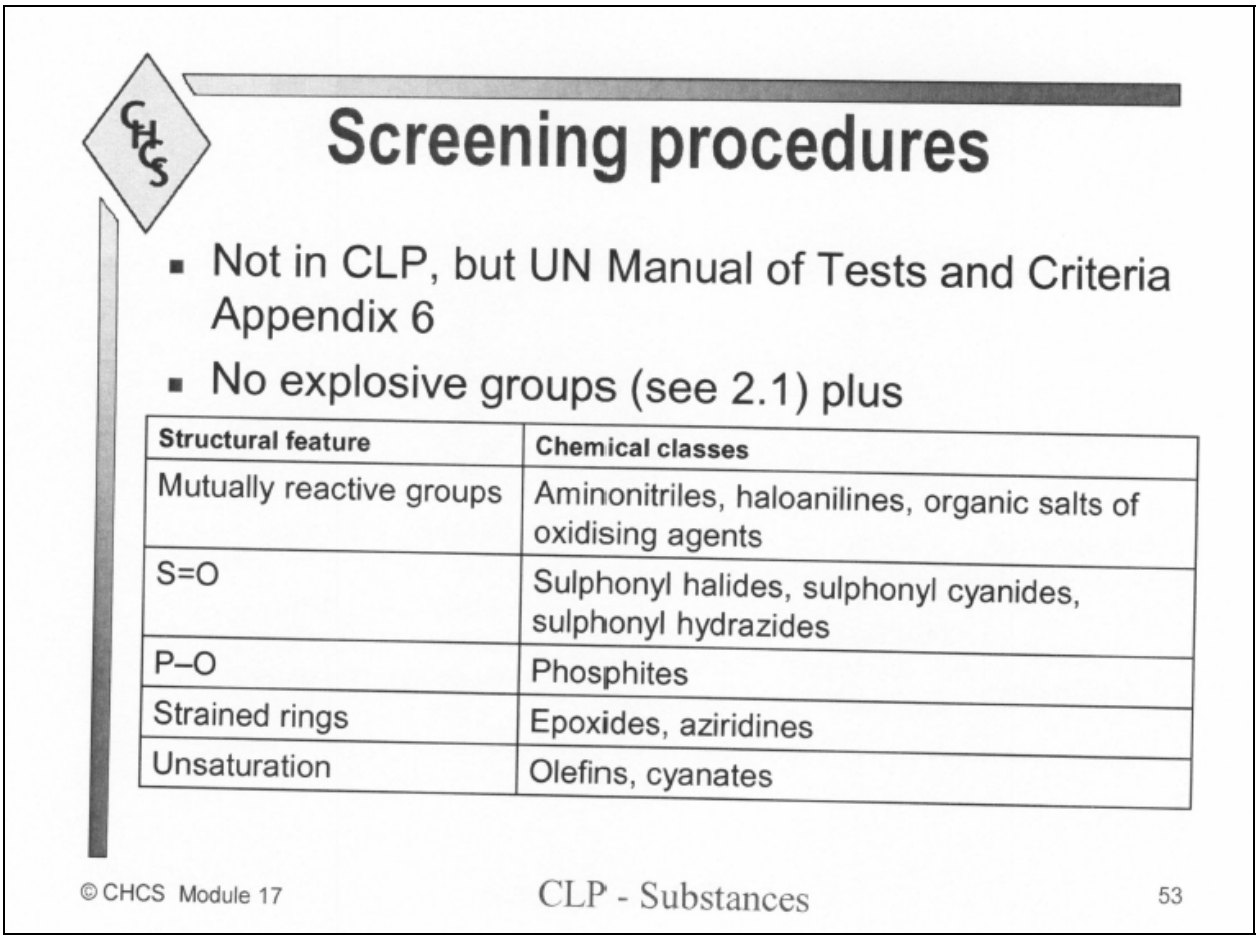
Table R.7.1-28 Chemical groups associated with explosive properties

Chemical group	Chemical Class
-C≡C- -C≡C-Metal -C≡C-Halogen	Acetylenic Compounds Metal Acetylides Haloacetylene Derivatives
	Diazo Compounds
-N=O -NO_2	Nitroso and Nitro Compounds,
R-O-N=O R-O-NO_2	Acyl or Alkyl Nitrites and Nitrates
	1,2-Epoxides
	Metal Fulminates or *aci*-Nitro Salts
	N-Metal Derivatives (especially heavy metals)
	N-Nitroso and N-Nitro Compounds
	N-Azolium Nitroimidates
	Azo Compounds
Ar-N=N-O-Ar	Arene Diazoates
$(ArN=N)_2O$, $(ArN=N)_2S$	Bis-Arenediazo Oxides and Sulfides
RN=N-NR'R''	Triazines
	High-nitrogen Compounds: e.g. Triazoles, Tetrazoles

Chemical group	Chemical Class
[1] ROOR', [2] 	Peroxy Compounds: [1] Alkyl hydroperoxides (R'=H), Peroxides (R'=organic); [2] Peroxo acids (R'=H), Peroxyesters (R'=organic)
[1] ROOMetal, [2] 	Metal peroxides, Peroxoacids salts
$-N_3$	Azides e.g. PbN_6, CH_3N_3
$^{-}O-C-N_2^{+}$	Arenediazonium oxides i.e. inner diazonium salts in which the counter ion is an oxide
Ar-N=N-S- Ar-N=N-S-Ar	Diazonium sulfides and derivatives, Arenediazo Aryl Sulfides
XO_n	Halogen Oxide: e.g. percholrates, bromates, etc
NX_3 e.g. NCl_3, $RNCl_2$	N-Halogen Compounds

Adapted from Bretherick (Bretherick's Handbook of Reactive Chemical Hazards 6th Ed., 1999, Butterworths, London)

Self-Reactive Substances



Screening procedures

- Not in CLP, but UN Manual of Tests and Criteria Appendix 6
- No explosive groups (see 2.1) plus

Structural feature	Chemical classes
Mutually reactive groups	Aminonitriles, haloanilines, organic salts of oxidising agents
S=O	Sulphonyl halides, sulphonyl cyanides, sulphonyl hydrazides
P–O	Phosphites
Strained rings	Epoxides, aziridines
Unsaturation	Olefins, cyanates

Licensed GreenScreen® Profilers

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