CARBON NANOFIBERS (CNFs)

(CAS #NA)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: July 1, 2021

ToxServices Review Date: July 1, 2026[1]

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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 Carbon Nanofibers (CNFs)

Carbon nanofibers (CNFs) are forms of carbon nanomaterials consisting of graphitic nanostructures of atomic layers arranged as stacked cups, cones, or plates. They are black crystalline particles that are highly insoluble in water due to their graphitic structure. CNFs have very high aspect ratios with diameters ranging from 5 to 100 nm and length from 5 to 100 μm. Three distinct structures for CNFs exist: 1) parallel or ribbon-like CNF (tubular), (2) platelet CNF, and (3) herringbone or fishbone type.

In this GreenScreen® assessment, ToxServices evaluated data on one type of CNFs, known as vapor-grown carbon nanofiber (VGCNF) with a herringbone structure, obtained from two different manufacturers, Pyrograf®-III and Showa Denko. VGCNF is produced by catalytic chemical vapor deposition, which is the main commercial technique for the synthesis of CNFs. The Pyrograf®-III and Showa Denko VGCNFs meet the World Health Organization (WHO) definition for respirable fiber (a particle longer than 5 μm, <3 μm in diameter, and with an aspect ratio (length/diameter) >3).

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

- Benchmark 1a
    - High Group I Human Toxicity (carcinogenicity-C)
- Benchmark 1d
    - Very High persistence-P + High Group II* Human Toxicity (systemic toxicity repeated exposure-STr*)
    - Very High P + High Group I Human Toxicity (C)

A data gap (DG) exists for endocrine activity-E[2]. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), CNFs meet requirements for a GreenScreen Benchmark™ Score of 1 despite the hazard data gap. In a worst-case scenario, if CNFs were assigned a High score for the data gap 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 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 MWCNTs' NAMs dataset (*in vitro* genotoxicity, and skin irritation tests) are identified. MWCNTs' Type II (extrapolation output) uncertainties include the limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, the potential non-applicability of the bacterial reverse mutation test to nanomaterials, and the limitation of the *in vitro* eye corrosion test **(**OECD Guideline 438) to identify substances classified as eye irritants (GHS Category 2A) or mild eye irritant (GHS Category 2B). The type II errors can be alleviated by the use of genotoxicity test batteries and *in vivo* data for eye irritation as there are no validated *in vitro* methods available for the direct identification of Category 2B eye irritants.

[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.

GreenScreen® Hazard Summary Table for CNFs

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*	*	*								
H	**M**	*L*	*L*	DG	**L**	**M**	**H**		*L*	**L**	*L*	**L**	*H*	**L**	*M*	*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 Carbon Nanofibers (CNFs) (CAS #NA)

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

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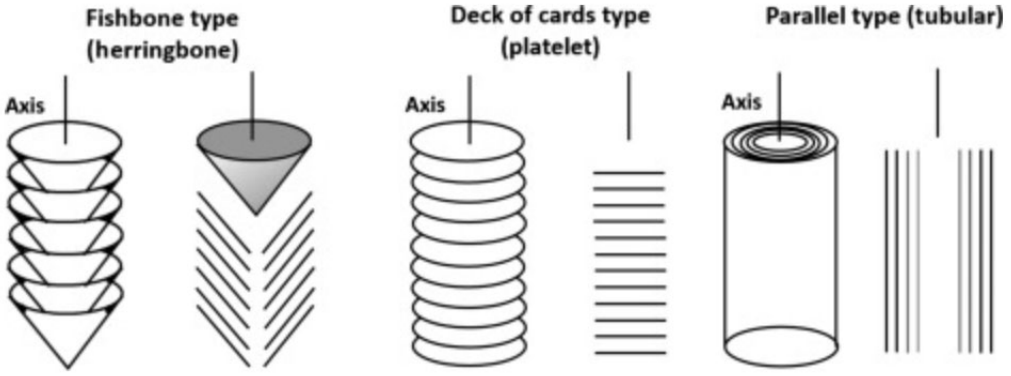
ToxServices Review Date: July 1, 2026[4]

Chemical Name: Carbon Nanofibers (CNFs)

CAS Number: A CAS number of 7782-42-5 is associated with carbon nanofibers (American Elements.2021) but this CAS refers to Graphite in the European Chemicals Agency (ECHA) Database (ECHA 2021a). ECHA, however, lists four different EC numbers for carbon nanofibers (unspecified) (EC #927-670-5) and its three types: carbon nanofibers herringbone-type (EC #924-992-8), carbon nanofibers platelet-type (EC # 929-224-5) and carbon nanofibers screw-type (EC #928-003-0). It is not clear if the screw-type is referring to the tubular form as introduced below.

Chemical Structure(s): CNFs are forms of carbon nanomaterials with diameters ranging from 5 to 100 nm and length from 5 to 100 μm. They consist of graphitic nanostructures of atomic layers arranged as stacked cups, cones, or plates. The structure and shape of CNFs may vary depending on the catalyst and production techniques. Three distinct structures for CNFs exist: 1) parallel or ribbon-like CNF (tubular), (2) platelet CNF, and (3) herringbone or fishbone type (Science Direct 2021). The three types of CNFs are shown below.



Schematic illustration of three different types of CNFs (Science Direct 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.

Also called: CNFs, Graphitized Carbon nano-fibers, Carbon nanofibres, Graphite conical platelet nanofibers, Graphitic carbon nanofibers, GNF, Pyrograf, vapor grown carbon fibers, vapor grown carbon nanofibers, VGCFs, VGCNFs, GNF-100, GNF-LSA, GNF-A, Pyrograf III, stacked-cup carbon nanotubes, carbon nanotube fibers, PR-19-XT-PS, PR-19-XT-LHT, PR-19-XT-HHT, PR-24-XT-PS, PR-24-XT-LHT, PR-24-XT-HHT, PR-25-XT-PS, PR-25-XT-LHT, PR-25-XT-HHT (American Elements.2021).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
The National Institute for Occupational Safety and Health (NIOSH) derived an exposure limit (REL) of 1 μg/m^3 as an 8-hour time-weighted average (TWA) for the respirable mass fraction of elemental carbon, single or multi-walled carbon nanotubes (SWCNTs or MWCNTs), and carbon nanofibers (NIOSH 2013). The derivation of this value for CNFs was based on data for one type of CNF called vapor-grown carbon nanofiber (VGCNF) with herringbone structure obtained from two different manufacturers, Pyrograf®-III and Showa Denko. ToxServices considered data on these two grades of VGCNFs in this assessment. Due to differences in manufacturing processes, these materials 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 CNF 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. Accordingly, the physicochemical characterization data for the two grades of VGCNTs used in this assessment are listed below:

1. Showa Denko VGCNF™: This substance has a chemical composition >99.5% carbon, with 0.03% oxygen and < 0.003% iron (NIOSH 2013). The average fiber length is 10 -20 μm, fiber diameter is 150 nm, specific surface area (SSA) is13.8 m^2/g, and aspect ratio is 10-500 (Showa Denko Undated). It has a REACH registration dossier with an EC number of 950-278-0 9 (ECHA 2021b).
2. Pyrograf®-III VGCNF of three different grades (PS, LHT, HHT): The chemical composition is > 98% of elemental carbon, up to 1.4% iron (Fe), and 0.8% sulfur (Pyrograf 2016a,b,c). The average fiber diameter is 125 to 150 nm, average fiber length is 50 to 100 μm, and the SSA is 24- 54 m^2/g (Sigma Aldrich 2021).

Limited data were identified for the above two grades of VGCNF. Therefore, surrogates were sought. ToxServices considered data on MWCNTs, particularly those with fiber form such as Nikkiso MWCNT and Mitsui MWCNT-7, to fill the data gaps. There are basically two different forms of MWCNT that are available commercially: one with a more rigid, long-fiber (asbestos-like) morphology and another one with a more tangled, short, low-density agglomerate form. Both the fiber form MWCNT and VGCNF are carbon nanomaterials and meet the World Health Organization (WHO) definition for respirable fiber (a particle longer than 5 μm, <3 μm in diameter, and with an aspect ratio (length/diameter) >3). In addition, available toxicity data on MWCNTs of fiber form and VGCNFs support that the hazard of these materials might be generically placed into the same hazard category (Oberdörster et al. 2015, Sigma Aldrich 2021). Therefore, ToxServices considered MWCNTs of fiber form as strong surrogates. For endpoints lacking data on fiber form MWCNTs, ToxServices considered data on the other form of MWCNTs (short and tangled) such as Graphistrength C100, and Hanwha CM-95 / CM-100. These were considered as weak surrogates. The physicochemical characterization data for the four types of MWCNTs used in this assessment are listed below:

1. Nikkiso MWCNT: It has an analytical purity of > 98% and contains calcium (Ca), aluminum (AL) and iron (Fe) as impurities. It is characterized by a tube diameter of 48 nm, a length of 0.94 μm (SD = 2.3), and a specific surface area of 69.4 m^2/g (OECD 2016, WHO 2017).

2. Mitsui / Hodogaya MWCNT-7: It has an analytical purity of > 95% and contains iron (Fe), chromium (Cr), and nickel (Ni) as impurities. It is characterized by an agglomerate/aggregate diameter of 1.5 μm (Geometric standard deviation (GSD) 1.67), a tube diameter of 70-170 nm, a length of 1-19 μm (>5 μm: 27.5%) and a specific surface area of 23 m^2/g (OECD 2016, WHO 2017).

3. Graphistrength C100: It is made of tightly bound agglomerates constituted with entangled MWCNTs. These agglomerates can be spherical, ovoid or irregular shaped. The median agglomerates diameter is in a range of 70 - 790 μm (ECHA 2021c). It has a purity of > 92%. It is characterized by an MMD of 416.2 μm (particle size), an average internal diameter of 4.8 nm, an average external diameter of 11.7 nm, an average length of 1097 nm, an average number of walls of 10, and a specific surface area of 212 m^2/g (OECD 2016, WHO 2017).

4. Hanwha CM-95 / CM-100: It has an analytical purity of 95% and contains iron (Fe), cobalt (Co) and aluminum oxide (Al_2O_3) as impurities. It is characterized by a tube diameter of 10 to 15 nm, a length less than 20 μm, and a specific surface area of 224.9 m^2/g (OECD 2016).

Identify Applications/Functional Uses:
Used in composite materials as a method of improving mechanical strength (Science Direct 2021). In addition, they are used in photocatalytic, energy devices, filtration, sensors, tissue engineering, and drug delivery (Science Direct 2021).

Known Impurities[5]:
Due to differences in manufacturing processes, CNFs may contain small amounts of the metallic catalyst such as Fe as impurities (IARC 2017). As described above, Pyrograf®-III VGCF of different grades (PS, LHT, HHT) contain iron and sulfur as impurities at concentrations up to 1.4 and 0.8%, respectively (Pyrograf 2016a,b,c).

GreenScreen® Summary Rating for CNFs[6,7 8,9]: CNFs 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 1a
  - High Group I Human Toxicity (carcinogenicity-C)
- Benchmark 1d
  - Very High persistence-P + High Group II* Human Toxicity (systemic toxicity repeated exposure-STr*)

[5] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.
[6] 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.
[7] See Appendix A for a glossary of hazard endpoint acronyms.
[8] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[9] 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.

- Very High P + High Group I Human Toxicity (C)

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), CNFs meet requirements for a GreenScreen Benchmark™ Score of 1 despite the hazard data gaps. In a worst-case scenario, if CNFs 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 CNFs

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*	*	*								
H	**M**	*L*	*L*	DG	**L**	**M**	**H**		*L*	**L**	*L*	**L**	*H*	**L**	*M*	*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 CNFs are inorganic nanomaterials that are persistent in the environment.

Introduction

CNFs are forms of carbon nanomaterials with diameters ranging from 5 to 100 nm and length from 5 to 100 µm. They have been synthesized through various methods such as chemical vapor deposition, electrospinning, templating, drawing, and phase separation. Catalytic chemical vapor deposition is considered the main commercial technique for the synthesis CNF as well as CNT. This technique produces CNFs known as VGCNF. CNFs are used in composite materials as a method of improving mechanical strength. In addition, they are used in photocatalytic, energy devices, filtration, sensors, tissue engineering, and drug delivery (Science Direct 2021).

ToxServices assessed CNFs 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).

CNFs are 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),[10] 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. CNFs are not listed in Pharos (Appendix C).

Hazard Statement and Occupational Control

VGCNFs from two different sources (Pyrograf®-III and Showa Denko) are associated with several Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements, as shown in Table 1, identified in their safety data sheets (Showa Denko 2016, Pyrograf 2016a,b,c). General personal protective equipment (PPE) recommendations are presented in Table 2 below. The NIOSH derived an REL of 1 μg/m^3 as an 8-hour TWA for the respirable mass fraction of elemental carbon, single or multi-walled carbon nanotubes, and carbon nanofibers (NIOSH 2013).

Table 1: GHS H Statements for VGCNFs (CAS #NA) (Showa Denko 2016, Pyrograf 2016a,b,c)

H Statement	H Statement Details
H332	Harmful if inhaled (Showa Denko VGCF™)
H373	May cause damage to organs (lung) through prolonged or repeated exposure (Showa Denko VGCF™)
H319	Causes serious eye irritation (Pyrograf®-III VGCF of different grades (PS, LHT, HHT))
H335	May cause respiratory irritation (Pyrograf®-III VGCF of different grades (PS, LHT, HHT))

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for CNFs (CAS #NA)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Wear eye protection, protective gloves, protective clothing, respiratory protection	Showa Denko 2016, Pyrograf 2016a,b,c	REL: 8h TWA: 1 μg/m^3 for the respirable mass fraction of elemental carbon, single or multi-walled carbon nanotubes, and carbon nanofibers	NIOSH 2013

NIOSH: National Institute for Occupational Safety and Health
REL: Recommended Exposure Limits
TWA: Time Weighted Average

Physicochemical Properties of VGCNFs

All CNFs are black crystalline particles that are highly insoluble in water. Due to differences in manufacturing processes, they can vary widely in their physiochemical properties which may affect their potential toxicity. The most important physicochemical characteristics which influence toxicity of CNFs 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 two grades of VGCNFs used in this assessment (Pyrograf®-III VGCF of different grades (PS, LHT, HHT), and Showa Denko VGCF™). Table 3 lists the other

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

physicochemical for these VGCNFs. As the CNFs meet the WHO definition of respirable fiber, inhalation is considered a primary route for human exposure.

Table 3: Physical and Chemical Properties of VGCNFs (CAS #NA)		
Property	**Value**	**Reference**
Molecular formula	C	ECHA 2021b, Pyrograf 2016a,b,c
SMILES Notation	[C]	ECHA 2021b, Pyrograf 2016a,b,c
Molecular weight	12	ECHA 2021b, Pyrograf 2016a,b,c
Physical state	Solid, nanomaterial form	ECHA 2021b, Pyrograf 2016a,b,c
Appearance	Black powder	ECHA 2021b, Pyrograf 2016a,b,c
Melting point	> 600°C (Showa Denko VGCF™) (GLP-compliant-OECD Guideline 102) 3,652 – 3,697°C (Pyrograf®-III VGCF of different grades (PS, LHT, HHT))	ECHA 2021b, Pyrograf 2016a,b,c
Boiling point	Not conducted as the melting point of the test substance is > 600°C and the substance undergoes oxidative decomposition in an air atmosphere at 840°C (Showa Denko VGCF™)	ECHA 2021b
Vapor pressure	Not available	ECHA 2021a
Water solubility	< 1 mg/L at 20°C and a pH of >= 5.4 - <= 6.6 (Showa Denko VGCF™) (OECD Guideline 105).	ECHA 2021b
Dissociation constant	Not available	ECHA 2021b
Density/specific gravity	Relative density = 2.12 g/cm³ (Showa Denko VGCF™) (OECD Guideline 109) Relative density = 2 - 20 lbs/ft³ (Pyrograf®-III VGCF of different grades (PS, LHT, HHT))	ECHA 2021b Pyrograf 2016a,b,c
Partition coefficient	Not applicable, as substance is inorganic	
Particle size	The average fiber length, fiber diameter and aspect ratio are 4.3 µm, 150 nm and 29, respectively. The percentage of particles ≤100 nm ranged from 8.0 % to 21.5 % (13.6 % average) (Showa Denko VGCF™) (OECD Guideline 109) The CNFs have average diameters ranging from 125 to 150 nm depending upon the grade, and have lengths ranging from 50 to 100 µm (Pyrograf®-III VGCF	ECHA 2021b Pyrograf 2016a,b,c

Table 3: Physical and Chemical Properties of VGCNFs (CAS #NA)		
Property	**Value**	**Reference**
	of different grades (PS, LHT, HHT))	
Structure	Graphite, herringbone type	Science Direct 2021
Bioavailability	Surrogate MWCNTs are not absorbed through skin and are estimated to have poor systemic absorption through the gastrointestinal tract.	ECHA 2021a

Toxicokinetics

No toxicokinetic data are available for CNFs. As they are similar to the class of MWCNTs, data on MWCNTs are considered, in particular those with fiber form. In general, MWCNTs are highly stable and chemically unreactive, absorption and metabolism in the body are not major concerns. The primary toxicokinetic considerations for MWCNTs are distribution and clearance in the respiratory tract. Measured data were available on the distribution of MWCNTs, and are described below:

- Absorption
  - OECD 2016
    - *Surrogate: MWCNTs as a class:* Oral absorption of MWCNT is not significant based on the results from acute toxicity studies in rats. In one study conducted with one type MWCNT where rats were given oral doses up to 2,000 mg/kg, no deaths occurred in spite of some toxic clinical signs observed. In the other study with Nikkiso MWCNT given up to 200 mg/kg, no effects were seen except for black feces.
- Distribution
  - OECD 2016
    - *Surrogate: MWCNTs as a class:* MWCNTs are expected to deposit in the lung and remain within the lungs for up to several months as indicated in several studies of MWCNTs when administered by inhalation, intratracheal instillation, ingestion or intravenous injection in rats.
    - *Surrogate: Nikkiso MWCNT:* In a 28-day inhalation repeated dose toxicity study with Nikkiso MWCNT, male Wistar rats were exposed to the test substance aerosol at a concentration of 0.37 mg/m^3 through whole body inhalation on 6 hours/day, 5 days/week. After the completion of inhalation exposure for 4 weeks, 10 rats from each group were dissected at 3 days, 1 month and 3 months. The lungs were isolated, and the amounts of MWCNT deposited in the lungs were determined by the X-ray diffraction method (XRD) and elemental carbon analysis (ECA). The average deposited amounts of MWCNT at 3 days after inhalation were determined as 68 µg/lung by XRD and 76 µg/lung by ECA. The calculated deposition fractions were 18% and 20% of inhaled MWCNT, respectively. The amount of retained MWCNT in the lungs until 3 months after inhalation decreased exponentially, and the calculated biological half-lives of MWCNT were 51 days (XRD) and 54 days (ECA).
    - *Surrogate: Mitsui MWNT-7:* In another inhalation study with Mitsui MWNT-7, mice were exposed to MWCNT dispersed aerosol for 2 hours a day for 5 days. In the peripheral alveolar space, single fibers were found phagocytized in alveolar macrophages.
- Metabolism
  - No data available.

- Excretion
    - OECD 2016
        - *Surrogate: Nikkiso MWCNT*: The main route for clearance from the body following oral uptake is via feces, as was seen in the 28-day oral repeated dose toxicity study (OECD Guideline 407) with Nikkiso MWCNT 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 (0.5, 5, 50 mg /kg/day). However, black feces in all treatment groups and greyish green or dark green colored contents in large intestine were observed in males and females at 5 mg/kg/day or more. This was due to the administered test item (black powder) and showed that the main elimination route from the body by oral (via gavage) administration is via feces.
- In summary, oral and dermal absorption of MWCNTs is assumed to be low. Inhalation absorption is assumed to be highly likely when the test substance has the ability to form aerosols. MWCNTs are expected to deposit in the lung and remain within the lungs for up to several months. MWCNT intratracheally instilled was translocated from the lung to lung associated lymph nodes, but there was no evidence of systematic distribution by inhalation. The main excretion pathway for absorbed MWCNT is expected to be via urine, and for unabsorbed MWCNT after ingestion is via feces.

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *H*
CNFs were assigned a score of High for carcinogenicity based on the EU-GHS proposed harmonized classification for the surrogates MWCNTs of fiber form to GHS Category 1B (presumed human carcinogen) supported by clear evidence of carcinogenicity in male and female F344 rats exposed to the surrogate MWCNT-7 aerosol by inhalation for 104 weeks. GreenScreen® criteria classify chemicals as a High hazard for carcinogenicity when they are classified to GHS Category 1B (CPA 2018b). The confidence in the score is low due to lack of information on the minimum physical parameters that would lead to the carcinogenic response of MWCNTs of fiber form.

- 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, Kasai et al. 2015
    - *Surrogate: Hodogaya MWCNT-7:* In a GLP-compliant two-year inhalation carcinogenicity study conducted according to OECD Guideline 451, male and female F344/DuCrlCrlj rats (50/sex/dose) were exposed by whole body inhalation to Hodogaya MWCNT-7 aerosol for 6 h/day, 5 days/week for 104 weeks at concentrations of 0, 0.02, 0.2, and 2 mg/m^3 using dry aerosol generation and exposure system. Treatment caused significant increases in lung carcinomas, mainly bronchioloalveolar carcinoma, and combined carcinomas and adenomas in males at 0.2 and 2 mg/m^3 and in females at 2 mg/m^3. Further, pre-neoplastic epithelial lesions were also significantly increased in males and females at these doses. The induction of carcinomas and combined carcinomas and adenomas was dose-dependent in male rats, and the induction of pre-neoplastic epithelial lesions was dose-dependent in both males and females. Induction of plural mesothelioma by exposure to MWCNT-7 was not observed in this study. However, simple mesothelial hyperplasia and focal fibrosis in the parietal pleura were found in rats exposed to 2 mg/m^3 MWNT-7. Study authors concluded that MWCNT-7

is carcinogenic to the lungs of male and female F344 rats, however no plural mesothelioma was observed.

- WHO 2017, IARC 2017
    - *Surrogate: MWCNTs:* The International Agency for Research on Cancer (IARC) Working Group reviewed the carcinogenicity of MWCNTs. No tumors were observed following intraperitoneal injection of short, tangled, low-density agglomerate form of MWCNT. However, studies with MWCNT of more rigid, long-fiber (asbestos-like) morphology (MWCNT-7) showed positive results: Accordingly, the IARC working group concluded that there was sufficient evidence for MWCNT-7, and limited evidence for the other two types of MWCNTs with dimensions similar to MWCNT-7. Mechanistic and other data in rodents provided evidence of translocation of three types of MWCNTs (including MWCNT-7) to the pleura. Additionally, inhalation of some MWCNTs or single-walled (SW) CNTs induced acute or persistent pulmonary inflammation, granuloma formation, fibrosis and bronchiolar or bronchioloalveolar hyperplasia in rodents. Studies in rodents and in cultured human lungs or mesothelial cells showed that MWCNTs, SWCNTs, or both, induce genetic lesions such as DNA strand breaks, oxidized DNA bases, mutations, micronucleus formation and chromosomal aberrations. SWCNTs and MWCNTs also perturb the cellular mitotic apparatus, including microtubules and centrosomes, in human lung epithelial cells. The IARC Working Group acknowledged that the above mechanisms are all relevant to humans. However, a majority of Working Group members did not consider the mechanistic evidence for carcinogenicity – especially concerning chronic endpoints – to be strong for any specific CNT. Furthermore, the lack of coherent evidence across the various distinct CNTs precluded generalization to other types of CNTs. Thus, MWCNT-7 was classified as possibly carcinogenic to humans (group 2B); and SWCNTs and MWCNTs excluding MWCNT-7 were categorized as not classifiable regarding their carcinogenicity to humans (group 3). *It is not clear why the two-year inhalation carcinogenicity study conducted with Hodogaya MWNT-7 that was described above was not considered by the IARC in its recent review.*
- ECHA 2021d
    - *Surrogate: MWCNTs:* MWCNTs of fiber form (fulfilling the WHO definition: diameter < 3 µm, fiber length > 5 µm and aspect ratio ≥ 3:1) have a proposed EU-GHS harmonized classification of Category 1B for carcinogenicity following inhalation exposure. The basis for the classification is not provided.
- Based on the weight of evidence, a score of High was assigned. No data are available for CNFs. The carcinogenicity of the surrogates, MWCNTs, has been reviewed by the IARC in which the Working Group classified the surrogate MWCNT-7 of the fiber form, 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 with the crucial steps for the formation of mesothelial carcinogens being the clearance from lung, and the entry into and clearance from mesothelium. All MWCNTs and CNFs are biopersistent. The longer and more rigid/needle-like a MWCNT/fiber the higher the carcinogenic risk, the shorter and the more bent, curved or waved the shape is, the lower their toxic and carcinogenic potency seems to be. 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.

However, the two-year inhalation carcinogenicity study conducted with Hodogaya MWCNT-7 of fiber form was not considered by the IARC in its review. In that study, there was clear evidence of carcinogenicity in male and female F344 rats exposed to MWCNT-7 aerosol by inhalation for 104 weeks. The study applied a route of exposure that is relevant to humans compared with the studies evaluated by IARC where the exposure routes used were not relevant for human exposure (e.g., injection in the intrascrotal cavity). With this confirmation of the carcinogenicity of inhaled MWCNT-7, Oberdörster and his co-workers proposed that the IARC classification is likely to change to: Probably carcinogenic to humans (Group 2A) (Oberdörster et al. 2015). According to GHS criteria, ToxServices classified MWCNT-7 to GHS Category 1B (presumed human carcinogen) as there is sufficient evidence to demonstrate animal carcinogenicity (UN 2019). This is consistent with the proposed EU-GHS harmonized classification of Category 1B for carcinogenicity for MWCNTs of fiber form. Both GHS Category 1B and IARC Group 2A correspond to a GreenScreen® score of High. Therefore, ToxServices relied on the results from the two-year inhalation carcinogenicity study with the MWCNT of fiber form (Hodogaya MWNT-7) as well as the proposed EU-GHS classification for such a form and assigned a score of High. The confidence in the score is low due to lack of information on the minimum physical parameters that would lead to the carcinogenic response of MWCNTs of fiber form.

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

CNFs were assigned a score of Moderate for mutagenicity/genotoxicity based on positive results for lung DNA damage seen in an *in vivo* assay conducted with one type of non-rigid form MWCNT (Hanwha CM-95) and some positive results for *in vitro* clastogenicity with two MWCNTs of the fiber form (Nikkiso and MWCNT-7) (although *in vivo* clastogenicity data for these MWCNTs were negative), leading the WHO Work Group to classify the entire MWCNT category to GHS Category 2 with a strong level of evidence. 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 high as it is based on strong evidence obtained from measured data of high quality for strong surrogates (several types of MWCNTs representing the two forms (rigid and long fiber / short and tangled)).

- 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 2021b
  - *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 TA 98, TA 100, TA 1535, and TA 1537, and *Escherichia coli* tester strain WP_2 *uvr* A were treated with Showa Denko VGCNF™ in dimethyl sulfoxide (DMSO) at concentration up to 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). *According to ECHA Guidance on nanomaterials, the in vitro bacterial mutagenicity testing is not recommended for these materials as the nanomaterials may not be able to cross the bacterial wall (ECHA 2020). Therefore, ToxServices did not weight heavily on the results from this assay.*
- OECD 2016, WHO 2017
  - <u>*Surrogate: Graphistrength C100 MWCNT:*</u> *In vitro*: Negative results for mutagenicity were obtained in a mammalian cell gene mutation test conducted according to OECD Guideline 476 with Graphistrength C100 MWCNT. L5178Y mouse lymphoma cells were exposed to

the test substance at concentrations up to 20 µg/mL for 3 hours or 24 hours with and without metabolic activation (S9 mix). No cytotoxicity and no increase in the mutation frequency was found at any of the tested dose levels. Vehicle and positive controls were valid.

- *Surrogate: Mitsui MWNT-7*: *In vitro*: Negative results for mutagenicity were obtained in a mammalian cell gene mutation test conducted according to OECD Guideline 476 with Mitsui MWNT-7. Chinese hamster lung cells (CHL/IU) were exposed to the test substance at concentrations ranging from 6.3 to 100 µg/mL for 48 hours without metabolic activation. No increase in the mutation frequency was induced at any of the tested dose-levels. Vehicle and positive controls were valid.
- *Surrogates: Nikkiso MWCNT and Mitsui MWNT-7*: *In vitro:* Positive results for clastogenicity were obtained in two chromosomal aberration tests conducted according to OECD Guideline 473 with two types of MWCNTs (Nikkiso MWCNT and Mitsui MWNT-7). CHL cells were exposed to the test substance at concentrations up to 100 µg/plate with and without metabolic activation. No significant increase in frequency of cells with structural aberrations was noted in any concentration tested with and without S9 mix. However, frequency of cells with numerical chromosomal aberrations were found to be slightly higher in Nikkiso MWCNT-treated cells and strongly higher in Mitsui MWNT-treated cells at 100 µg/mL without S9.
- *Surrogate: Graphistrength C100 MWCNT:* *In vitro:* Negative results for clastogenicity were obtained in a chromosomal aberration test conducted according to OECD Guideline 473 with Graphistrength C100 MWCNT. Human lymphocytes were exposed to the test substance at concentrations up to 50 µg/mL with and without S9 mix. Since precipitation occurred at concentrations of 25 µg/mL or more, observation of chromosomal aberrations was conducted up to 12.5 µg/mL. No significant increase in frequency of cells with structural aberrations was noted in any concentration tested with and without S9 mix.
- *Surrogate: Mitsui MWNT-7*: *In vitro:* Positive results for clastogenicity were obtained in a chromosomal aberration test conducted according to OECD Guideline 473 with Mitsui MWNT-7. CHL/IU were exposed to the test substance at concentrations ranging from 1.3 to 80 µg/mL for 24 hours or at concentrations ranging from 0.078 to 5.0 µg/mL for 48 hours without S9 mix. Structural chromosomal aberrations were not observed. However, significantly increased number of cells with numerical aberrations (polyploidy) was observed at concentrations of 5 µg/mL or more in 24 hours treatment and at concentrations of 1.3 and 5.0 µg/mL in 48 hours treatment.
- *Surrogate: Hanwha CM-95 MWCNT*: *In vitro:* Negative results for clastogenicity were obtained in a GLP-compliant chromosome aberration test conducted according to OECD Guideline 473 with Hanwha CM-95 MWCNT. Chinese hamster ovarian fibroblasts (CHO-K1) were exposed to the test substance at concentrations up to 6.25 µg/mL for 6 hours and 24 hours without S9, or at concentrations up to 25 µg/mL for 6 hours. There was no evidence of induction of chromosomal aberrations (structural and numerical).
- *Surrogate: Mitsui MWNT-7*: *In vitro:* Positive results for clastogenicity were obtained in an *in vitro* mammalian cell micronucleus test for Mitsui MWNT-7 using CHL/IU cells at concentrations up to 5.0 µg/mL for 48 hours without metabolic activation. A significant increase in the numbers of bi-nucleated and multi- nucleated cells without micronucleus induction was seen.
- *Surrogates: Nikkiso MWCNT and Mitsui MWNT-7*: *In vivo*: Nikkiso MWCNT and Mitsui MWNT-7 were negative in two *in vivo* micronucleus tests conducted according to OECD Guideline 474 using male and female ICR mice (6/dose) that received oral doses of the test substances via gavage at 0, 5, 10 and 20 mg/kg/day, once daily for 2 consecutive days.

Animals were sacrificed after 24 hours. There were no increases in micronuclei in the bone marrow. Vehicle and positive controls were valid.

- <u>*Surrogate: Hanwha CM-95 MWCNT*</u>: *In vivo*: In another micronucleus assay conducted according to OECD Guideline 474, male ICR mice (6 animals/dose) were treated intraperitoneally with Hanwha CM-95 MWCNT at doses of 0 (vehicle: DPPC), 12.5, 25 and 50 mg/kg. Animals were sacrificed after 24 hours. There were no increases in micronuclei in the bone marrow.
- <u>*Surrogate: Hanwha CM-95 MWCNT*</u>: *In vivo*: Positive results for lung DNA damage were seen in a non-guideline *in vivo* comet assay conducted with Hanwha CM-95 MWCNT. Male SD rats (10 animals/dose) were exposed to the test substance by whole body inhalation at concentrations of 0, 0.16, 0.34 or 0.94 mg/m^3 for 6 hours/day for 5 days. Animals were sacrificed by the end of exposure period or one month later, and the lung cells were isolated. A single cell gel electrophoresis assay was conducted to determine DNA damage in lung cells. The Olive Tail Moment (OTM) used as a parameter of comet assay was analyzed using fluorescent micrometer and image program. Treatment caused significant increase in OTM in the group exposed to the highest concentration (148% of the negative control) at the end of exposure. This elevation of OTM in the highest concentration groups was still observed (128% of the negative control) one month post exposure.
- <u>*Surrogates: MWCNTs as a class*</u>: Based on the above studies, the WHO Working Group concluded that most CNTs if exposed with a dispersed CNT structure can be genotoxic. MWCNTs with agglomerated/aggregated form like Graphistrength were negative for genotoxicity in *in vitro* assays, but MWCNTs having rigid and fiber structure such as Nikkiso and MWCNT-7 were positive in *in vitro* genotoxicity tests. Further, *in vivo* micronucleus tests conducted by oral and intraperitoneal administrations were negative, but a comet assay of the lung cells that were actually exposed by inhalation were positive with Hanwha CM-95 MWCNT. Taken together with the limitation of *in vivo* dosing data, the WHO Working Group classified the entire MWNCTs category as GHS Category 2 for germ cell mutagenicity with high confidence.

Reproductive Toxicity (R) Score (H, M, or L): *L*

CNFs 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 the surrogate MWCNT of short and tangled form (Graphistrength C100). 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 for a weak surrogate obtained 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.
- OECD 2016, WHO 2017
  - <u>*Surrogates: MWCNTs as a class*</u>: No reproductive toxicity studies are available for MWCNTs. In repeated dose toxicity studies including inhalation studies for 2 to 13 weeks and an oral 28-day study, no effects were seen in the reproductive organs of either sex in rats.
- ECHA 2021c
  - <u>*Surrogate: Graphistrength C100 MWCNT: Inhalation*</u>: In a GLP-compliant reproduction/developmental toxicity screening study conducted according to OECD

Guideline 421, male and female RccHan™ WIST rats (10/sex/dose) were exposed by nose only inhalation to Graphistrength C100 MWCNT for 6 h/day, 7 days/week at concentrations of 0, 0.285, 1.41, and 5.6 mg/m^3 (the maximum achievable dose). 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 ovaries 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, anogenital 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. The study authors identified the reproductive toxicity NOAEC as 5.6 mg/m$^{3/}$6h/day, the highest dose tested (Klimisch 1, reliable without restriction).

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *L*
CNFs were assigned a score of Low for developmental toxicity based on the lack of developmental effects in an OECD Guideline 414 study with the surrogate, MWCNT of short and tangled form (Hanwha CM-95). GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate data are available and negative, and they are not GHS classified (CPA 2018b). The confidence in the score is low as it is based on measured data of high quality for a weak surrogate (short and tangled MWCNT).

- 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 2021c
  - <u>*Surrogate: Graphistrength C100 MWCNT*</u>: *Inhalation*: In the previously described GLP-compliant reproduction/developmental toxicity screening conducted according to OECD Guideline 421, male and female RccHan™ WIST rats (10/sex/dose) were exposed by nose only inhalation to Graphistrength C100 for 6 h/day, 7 days/week at concentrations of 0, 0.285, 1.41, and 5.6 mg/m^3 (the maximum achievable dose). 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 ovaries 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, anogenital distance, nipple retention (male pups), and external and internal abnormalities. There were no embryotoxic or teratogenic effects observed with treatment. She study authors identified the developmental toxicity NOAEC as 5.6 mg/m$^{3/}$6h/day, the highest dose tested (Klimisch 1, reliable without restriction).
- WHO 2017, OECD 2016
  - <u>*Surrogate: Hanwha CM-95 MWCNT*</u>: *Oral:* In a prenatal developmental toxicity study conducted according to OECD Guideline 414, pregnant female SD rats (12/group) were administered Hanwha CM-95 MWCNT at doses of 0, 40, 200 or 1,000 mg/kg/day by gavage

on gestational day 6 (GD6) until GD19. All dams were sacrificed on GD20, and the fetuses were morphologically examined for external, visceral or skeletal anomalies. No embryotoxicity or teratogenicity was observed with treatment. Treatment caused maternal toxicity as characterized by a decrease in thymus weight in 1,000 mg/kg/day group. Authors assigned a NOAEL 1,000 mg/kg/day for developmental toxicity, which was the highest dose tested. The NOAEL for maternal toxicity was 200 mg/kg/day.

- *Surrogate: Mitsui MWNT-7*: *Intratracheal/intraperitoneal*: In another study performed to examine a teratogenic potential of MWCNTs compulsorily injected into the body prenatally, Mitsui MWNT-7 was suspended in 2% CMC solution and given to pregnant ICR mice either intraperitoneally at doses of 2, 3, 4 or 5 mg/kg or intratracheally at doses of 3, 4 or 5 mg/kg on GD9. Treatment caused various types of malformations in all groups receiving the test substance intraperitoneally, while such malformations were observed in groups given 4 or 5 mg/kg in the intratracheal study. In addition, the number of litters having fetuses with external malformations and skeletal malformations were both increased in a dose dependent manner. The authors therefore suggested that MWCNT has a potential of teratogenicity. However, the WHO Working Group considered this study inappropriate for GHS classification purposes since the routes of exposure are not recommended by GHS criteria; in addition, the doses used in this study, in terms of large volumes per mass, were so extreme that this alone may have caused the teratogenicity.
- *Surrogates: MWCNTs as a class*: Based on the results from the standard OECD Guideline 414 study with Hanwha CM-95 MWCNT, no developmental toxicity hazard classification was assigned for MWCNTs in the WHO report.

Endocrine Activity (E) Score (H, M, or L): DG
CNFs 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
CNFs were assigned a score of Low for acute toxicity based on an oral LD_{50} > 2,000 mg/kg, and an inhalation LC_{50} > 1.78 mg/m^3 (the maximum achievable concentration) for one type of CNFs (Showa Denko VGCNF™). 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 high as it is based on measured data of good 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 2021b
  - *Oral*: LD_{50} (Wistar female rats) > 2,000 mg/kg for Showa Denko VGCNF™ (GLP-compliant, OECD Guideline 420) (Klimisch 1, reliable without restriction).

  - *Inhalation:* LC_{50} (Sprague-Dawley rats) > 1.87 mg/L/4h dust (maximum technically attainable concentration) for Showa Denko VGCNF™ (GLP-compliant, OECD Guideline 403). Only one death occurred in a group of ten rats exposed to a mean maximum attainable atmosphere concentration of 1.87 mg/L in air for 4 hours (Klimisch 1, reliable without restriction).
- Showa Denko 2016
  - *Inhalation:* A safety data sheet for Showa Denko VGCNF™ classified it to GHS Category 4 for inhalation acute toxicity with a hazard statement of H332: Harmful if inhaled with the reported LC_{50} of > 1.87 mg/L/4h. *According to GHS criteria, when the inhalation LC_{50} is greater than the maximum attainable atmosphere concentration, then no classification for acute inhalation toxicity is warranted. Therefore, ToxServices disregarded the assigned classification for Showa Denko VGCNF™ in its SDS and relied on the measured LC_{50} which appears to be from the same study as reported in the ECHA dossier above.*

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

CNFs were assigned a score of Moderate for systemic toxicity (single dose) based on ToxServices classifying one type of CNFs (Showa Denko VGCNF™) to GHS Category 3 (respiratory irritation) following single inhalation exposure supported by the same classification assigned for another VGCNFs, Pyrograf®-III VGCNF of different grades, in their safety data sheets. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (single dose) when they are classified to GHS Category 3 (respiratory irritation) (CPA 2018b). The confidence in the score is high as it is based on measured data of good 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 2021b
  - *Oral:* In a GLP-compliant acute oral toxicity study conducted according to OECD Guideline 420, five Wistar female rats were administered Showa Denko VGCNF™ in arachis oil at a single dose of 2,000 mg/kg by gavage. Animals were observed for 14 days following administration and necropsied for toxicity evaluation. No deaths occurred and no clinical signs of toxicity were seen. Body weight development was not affected. There were no abnormal gross findings at necropsy. Authors identified an oral LD_{50} of > 2,000 mg/kg (Klimisch 1, reliable without restriction).
  - *Inhalation:* In a GLP-compliant acute inhalation toxicity study conducted according to OECD Guideline 403, Sprague-Dawley rats (5/sex/dose) were exposed to Showa Denko VGCNF™ dust via nose-only inhalation at a concentration of 1.87 mg/L, which was the maximum attainable concentration, for four hours. Animals were observed for 14 days. The respiratory tract was subject to a detailed macroscopic examination for signs of irritancy or local toxicity. One male died before scheduled sacrifice. Treated animals exhibited clinical signs of toxicity such as increased respiratory rate, labored respiration, noisy respiration, hunched posture, pilo-erection, fur staining by the test material and wet fur. Abnormally dark lungs and pale patches on the lungs were noted in all animals that survived until Day 14. The animal that died during the course of the study showed abnormally dark lungs with dark patches. Body weight development was not affected. Authors identified an inhalation LC_{50} of > 1.87 mg/L/4h, as this is the maximum technically attainable concentration. *Based on the signs of respiratory irritation (increased respiratory rate, labored respiration, noisy*

respiration), ToxServices classified Showa Denko VGCNF™ to GHS Category 3 for systemic toxicity following single exposure (respiratory irritation).

- Pyrograf 2016a,b,c
  - Pyrograf®-III VGCNF of different grades (PS, LHT, HHT) are classified in their safety data sheets to GHS Category 3 for Specific target organ toxicity – single exposure (Respiratory system) with a hazard statement of H335: May cause respiratory irritation.
- WHO 2017
  - *Surrogate: MWCNTs as a class:* No acute toxicity studies using normal exposure routes were identified. Other studies using intratracheal instillation, pharyngeal aspiration and intraperitoneal injection of MWCNTs 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 MWCNTs under GHS.

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

CNFs were assigned a score of High for systemic toxicity (repeated dose) based on respiratory effects seen in a 90-day inhalation repeated dose toxicity study with one type of CNFs (Showa Denko VGCNF™) with a LOAEC of 1.78 mg/m^3/6h/day, classifying to GHS Category 1. 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 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 authoritative lists for this endpoint.
- ECHA 2021b, NIOSH 2013, DeLorme et al. 2012
  - *Inhalation*: In a GLP-compliant subchronic inhalation repeated dose toxicity study conducted according to OECD Guideline 413, male and female Crl:CD(SD) rats (10/sex/dose) were exposed by nose only inhalation to Showa Denko VGCNF™ at concentrations of 0, 0.54, 2.5 and 25 mg/m^3 for 6 hours/day, 5 days/week for 13 weeks. Guideline-recommended examinations were fully performed. Treatment caused respiratory effects as characterized by the significant increase of the wet lung weights in male rats at 25 mg/m^3 and in female rats at 2.5 and 25 mg/m^3 at 1-day post-exposure with the lung weights remaining elevated in each sex in the high exposure group at 3 months post-exposure. In addition, a concentration-related accumulation of fibers within alveolar macrophages was seen, and at the two highest exposure concentrations, subacute to chronic inflammation of the terminal bronchiole and alveolar duct areas of the lungs was observed. After a three-month recovery period, the fiber-laden alveolar macrophages still persisted in the lungs. However, the centriacinar inflammation was less severe. Based on the slight subacute to chronic inflammation of the terminal bronchiole and alveolar duct areas of the lungs with some thickening of interstitial walls and hypertrophy/hyperplasia of type II pneumocytes in rats exposed to 2.5 mg/m^3, authors assigned a NOAEC of 0.54 mg/m^3 for systemic toxicity (equivalent to 0.38 mg/m^3/6h/day[11]). *The LOAEC of 2.5 mg/m^3 (equivalent to 1.78 mg/m^3/6h/day[12]) is below the GHS guideline value of 20 mg/m^3/6h/day for Category 1 (dust)*

[11] Converting exposure period 5days/week to daily = 0.54 mg/m^3 x 5 / 7(days) = 0.38 mg/m^3/day
[12] Converting exposure period 5days/week to daily = 2.5 mg/m^3 x 5 / 7(days) = 1.78 mg/m^3/day

for a 90-day study. Therefore, Showa Denko VGCNF™ is classified to GHS Category 1 for systemic toxicity repeated dose.

  - Based on the results from the above study, the authors of REACH dossier classified Showa Denko VGCNF™ to GHS Category 1 for systemic toxicity following repeated inhalation exposure with the lung identified as the target organ.
- ECHA 2021d
  - *Surrogate: MWCNTs as a class:* MWCNTs of fiber form (fulfilling the WHO definition: diameter < 3 µm, fibre length > 5 µm and aspect ratio ≥ 3:1) have a proposed EU-GHS harmonized classification of Category 1 for systemic toxicity following repeated exposure. The basis for the classification is not provided.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): DG
MWCNTs 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*
CNFs 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 28-day repeated dose toxicity study performed with the surrogate MWCNT of short and tnagled form (Graphistrength C100). 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 low as it is based on data for a weak surrogate.

- 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 2021b
  - *Surrogate: Graphistrength C100 MWCNT: Oral*: In a GLP-compliant repeated dose toxicity study conducted according to OECD Guideline 407, male and Female Sprague-Dawley rats (5 /sex/dose) were administered Graphinstrength C100 MWCNT in diet at concentrations of 100, 1,000 and 10,000 ppm for 4 weeks, corresponding to mean achieved dose levels of 10, 95 and 951 mg/kg/day for males and 11, 105 and 1,073 mg/kg/day for females, respectively, as calculated by the study authors. 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 duration-adjusted GHS Category 2 cut-off value of 300 mg/kg/day[13] for a 28-day study. Therefore, the test substance is not classified per GHS.*

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): L
CNFs were assigned a score of Low for skin sensitization based on negative results in skin sensitization studies performed with one type of CNFs (Showa Denko VGCF™) and two MWCNTs of the fiber form

[13] 100 mg/kg/day x 90 days /28 days = 300 mg/kg/day

(Nikkiso, and Mitsui MWNT-7). 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 chemical and strong surrogates.

- 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 2021b
  - Showa Denko VGCF™ was not sensitizing in a GLP-compliant guinea pig maximization test (GPMT) conducted according to OECD Guideline 406. Male Hartley guinea pigs (10/dose) were intradermally and epicutaneously (occlusive) induced with 5 or 15% of the test substance in olive oil for 24 hours each with a 3-week rest phase. The animals were then challenged with 0.01%. Some positive reactions were observed in treated animals, but these were attributed to the vehicle used, olive oil, which is well-known for its permeation enhancement properties. The authors concluded that Showa Denko VGCF™ is not sensitizing under the conditions of the assay (Klimisch 1, reliable without restriction).
- OECD 2016, WHO 2017
  - *Surrogates: Nikkiso MWCNT and Mitsui MWNT-7*: Both Nikkiso MWCNT and Mitsui MWNT-7 were non-sensitizing to the skin of male guinea pigs (n = 20) when tested in a Buehler test conducted according to OECD Guideline 406. The test substance in olive oil was epicutaneously applied to male Hartley guinea pigs once a week, three times in total (day 0, 7 and 14) in the induction phase. Two weeks after the last induction, elicitation exposure with 1% (Nikkiso MWCNT) or 2% (Mitsui MWNT-7) in petrolatum was epicutaneously applied for 6 hours. No clinical signs or changes in body weight gain were observed in any group. No positive reactions were seen.
  - *Surrogate: MWCNTs as a class*: Based on the above results, no skin sensitization hazard classification was assigned for MWCNTs in the WHO report with the level of evidence being considered as strong.

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): *L*

CNFs 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
  - *Surrogate: MWCNTs as a class*: MWCNTs are not respiratory sensitizers based on negative results in skin sensitization studies performed with three types of MWCNTs. Based on this, no respiratory sensitization hazard classification was assigned for MWCNTs in the WHO report with the level of 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 CNFs 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 CNFs, they are not expected to be respiratory sensitizers.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): L
CNFs were assigned a score of Low for skin irritation/corrosivity based on negative results in a dermal irritation study performed with one type of CNF (Showa Denko VGCF™) supported by negative data for surrogates (Mitsui MWCNT-7, and Nikkiso MWCNT). 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 high as it is based on measured data of good quality for the target chemical and strong surrogates.

- 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 2021b
  - In a GLP-compliant dermal irritation study conducted according to OECD Guideline 404, 0.5 g Showa Denko VGCNF™ moistened with water was applied to the clipped skin of three male New Zealand white rabbits for 4 hours under semiocclusive conditions. Animals were observed for up to three days after the exposure period. None of the animals showed erythema or edema at 1, 2, 48, or 72 hours (mean scores is 0). Accordingly, the test substance was determined to be non-irritating under the conditions of the assay (Klimisch 1, reliable without restriction).
- OECD 2016, WHO 2017
  - *Surrogate: Nikkiso MWCNT and Mitsui MWCNT-7*: In two dermal irritation studies conducted according to OECD Guideline 404, 0.5 g of two types of MWCNTs in the fiber form (Mitsui MWCNT-7, and Nikkiso MWCNT) were applied to the shaved skin of three male New Zealand white rabbits under occlusive conditions for 4 hours. Mitsui MWCNTs did not cause any skin irritation. The value of the primary irritation index (P.I.I) was 0.0. Treatment with Nikkiso MWCNT caused slight skin irritation as erythema was observed at day 4-5 and 24-48 hours respectively. However, at day 6-8 and 72 hours, there were no longer any signs of erythema present. The value of P.I.I was 0.6 for Nikkiso MWCNT.
  - *Surrogate: MWCNTs as a class*: Based on the results from the above studies with several types of MWCNTs, no skin irritation hazard classification was assigned for MWCNTs in the WHO report with the level of evidence being considered as strong.

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): *H*
CNFs were assigned a score of High for eye irritation/corrosivity based on one type of CNFs (Pyrograf®-III VGCNF) of different grades being classified in their safety data sheets 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 mixed results with different types of CNFs and due to lack of supporting measured data on Pyrograf®-III VGCNF grades.

- 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 2021b

  - Showa Denko VGCNF™ was not corrosive to the eye when tested in a GLP-compliant *in vitro* ocular irritation test conducted according to OECD Guideline 438 (Isolated Chicken Eye Test (ICE) Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage). (Klimisch 1, reliable without restriction).
- Pyrograf 2016a,b,c
  - Pyrograf®-III VGCNF of different grades (PS, LHT, HHT) are classified in their safety data sheets to GHS Category 2A for eye irritation with a hazard statement of H319 Causes serious eye irritation.
- OECD 2016
  - *Surrogate: Nikkiso MWCNT*: A 0.25% suspension of Nikkiso MWCNT was slightly irritating to the eye when tested in an ocular irritation test similar to OECD Guideline 405. An amount of 0.1 mL of 0.25% (Nikkiso MWCNT) suspension in a minimum amount of olive oil was instilled in the conjunctivae of the eye of three male New Zealand White rabbits for one second, and the eye was rinsed 1 hour later. At 1, 24, 48 and 72 hours, the eyes were observed and scored. Treatment caused redness of conjunctivae (score=1) in all 3 rabbits at 1 hour after instillation, with effects being fully reversible within 24 hours.
  - *Surrogate: Mitsui MWNT-7*: In another ocular irritation test similar to OECD Guideline 405, 1.0% suspension of Mitsui MWNT-7 in olive oil was not irritating to the eye of three male New Zealand White rabbits when instilled at an amount of 0.1 mL for one second.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): L
CNFs 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 one type of CNFs (Showa Denko VGCNF™) 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 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 2021b
  - 96-hour LL_{50} (*Cyprinus carpio*, fish) > 100 mg/L for Showa Denko VGCF™ (OECD Guideline 203, GLP-compliant). No abnormal behaviour and no mortality were observed (Klimisch 2, reliable with restrictions).
  - 48-hour EL_{50} (*Daphnia magna*, invertebrate) > 100 mg/L (immobilization) for Showa Denko VGCF™ (GLP-compliant, OECD Guideline 202). No abnormal behaviour and no mortality were observed (Klimisch 2, reliable with restrictions).
  - 72-hour EL_{50} (*Pseudokirchneriella subcapitata*, green algae) for growth rate > 100 mg/L for Showa Denko VGCF™ (GLP-compliant, OECD Guideline 201) (Klimisch 2, reliable with restrictions).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *M*
CNFs were assigned a score of Moderate for chronic aquatic toxicity based on a measured NOEC value of 3.2 mg/L in fish for a surrogate Nikkiso MWCNT. GreenScreen® criteria classify chemicals as a Moderate hazard for chronic aquatic toxicity when the chronic aquatic toxicity values are > 1.0 mg/L

and < 10 mg/L (CPA 2018b). The confidence in the score is reduced as the reported NOEC is above the water solubility of CNFs.

- 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 2021b
  - 72-hour NOELR (*P. subcapitata*, green algae) for growth rate = 100 mg/L for Showa Denko VGCNF™ (GLP-compliant, OECD Guideline 201) (Klimisch 2, reliable with restrictions).
- OECD 2016
  - <u>*Surrogate: Nikkiso MWCNT*</u>: 14-day NOEC (*Oryzias latipes*, fish) = 3.2 mg/L, 14-day LOEC = 10 mg/L (GLP-compliant, OECD Guideline 204). The reported concentrations are nominal, and analytical confirmation of the concentrations were not carried out. Study authors used the surfactant Tween 80 to aid the dispersion of the test substance in the water. The NOEC and LEC were based on weight gain and/or mortality.
  - <u>*Surrogate: Nikkiso MWCNT*</u>: 21-day NOEC (*D. magna*, invertebrate) = 0.32 mg/L, the highest tested concentration (GLP-compliant, OECD Guideline 211).

<u>Environmental Fate (Fate)</u>

Persistence (P) Score (vH, H, M, L, or vL): *vH*

CNFs were assigned a score of Very High for persistence based on one type of CNFs (Showa Denko VGCNF™) not being readily biodegradable when tested according to OECD Guideline 301 B and based on expert judgment that CNFs 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 low due to lack of measured half lives data in the environmental compartments.

- 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 2021b
  - Showa Denko VGCNF™ was not readily biodegradable when tested in a GLP-compliant biodegradation test conducted according to OECD Guideline 301 B (CO2 Evolution Test). Activated sludge, domestic, non-adapted were exposed to VGCNF™ at concentration of 12 mg/L for 28 days. A biodegradation rate of 3% was achieved by the end of the 28-day exposure period (Klimisch 1, reliable without restriction).

Bioaccumulation (B) Score (vH, H, M, L, or vL): *vL*

CNFs were assigned a score of Very Low for bioaccumulation based on expert judgment related to their large molecular diameters (typically > 1.5 nm). 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 low as it is based on expert judgment and 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.
- Aschberger et al. 2016
  - <u>*Surrogate: MWCNTs as a class*</u>: MWCNTs have low potential for bioaccumulation due to their high molecular weight and diameter. Accordingly, Aschberger et al. assigned a

GreenScreen® score of Low for bioaccumulation. The confidence in the score was low as some studies indicated uptake in plants and fish, and therefore further investigations into that issue are warranted.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*
CNFs were assigned a score of Low for reactivity based on HMIS and NFPA reactivity rating of 0 for one type of CNFs (Pyrograf®-III VGCNF of different grades) 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 2021b
  - Showa Denko VGCNF™ 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.
  - Showa Denko VGCNF™ is not considered to have explosive properties as it does not contain any functional groups associated with explosive or self-reactive properties (See Appendix D).
- Pyrograf 2016a,b,c
  - Safety data sheets for Pyrograf®-III VGCNF of different grades (PS, LHT, HHT) have a reactivity rating of 0 from the NFPA and HMIS; which correspond to "Normally stable, even under fire exposure conditions, and is not reactive with water" [14] and "Materials that are normally stable, even under fire conditions, and will not react with water, polymerize, decompose, condense, or self-react. Non-explosives" [15], respectively.

Flammability (F) Score (vH, H, M, or L): L
CNFs were assigned a score of Low for flammability based on one type of CNFs (Showa Denko VGCNF™) not being classified as a flammable solid when tested according to UN Test N.1 (Test method for readily combustible solids), supported by the HMIS and NFPA flammability rating of 0 for another VGCNF of different grades (Pyrograf®-III). 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 high as it is based on measured data of good 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 2021b
  - In a preliminary screening test for flammability conducted according to the UN Manual of Tests and Criteria: Test N.1 (Test method for readily combustible solids), Showa Denko VGCNF™ was not flammable as it did not ignite and propagate combustion either by

[14] https://www.fm.colostate.edu/files/forms/safety/CH-23.NFPA.ratings.pdf
[15] http://www.ilpi.com/msds/ref/hmis.html

burning with flame or smoldering along 200 mm of the powder train within the 2 minutes test period (Klimisch 1, reliable without restriction).
    - In a test method for pyrophoric solids) conducted according to UN Manual of Tests and Criteria: Test N.2, Showa Denko VGCNF™ did not show any pyrophoric properties (Klimisch 1, reliable without restriction).
- Pyrograf 2016a,b,c
    - Safety data sheets for Pyrograf®-III VGCNF of different grades (PS, LHT, HHT) have a flammability rating of 0 from the NFPA and HMIS which corresponds to "Materials that will not burn"[16], and "materials that are normally stable and will not burn unless heated"[17], respectively.

[16] https://www.fm.colostate.edu/files/forms/safety/CH-23.NFPA.ratings.pdf
[17] http://www.ilpi.com/msds/ref/hmis.html

Use of New Approach Methodologies (NAMs)[18] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* tests for genotoxicity 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 MWCNTs' NAMs dataset (*in vitro* genotoxicity, and skin irritation tests) are identified. MWCNTs' Type II (extrapolation output) uncertainties include the limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, the potential non-applicability of the bacterial reverse mutation test to nanomaterials, and the limitation of the *in vitro* eye corrosion test **(**OECD Guideline 438) to identify substances classified as eye irritants (GHS Category 2A) or mild eye irritant (GHS Category 2B). The type II errors can be alleviated by the use of genotoxicity test batteries and *in vivo* data for eye irritation as there are no validated *in vitro* methods available for the direct identification of Category 2B eye 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, and Skin Irritation**: No Type I uncertainty is identified on using the *in vitro* genotoxicity, 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 aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[19]. The mammalian cell gene mutation assay (as defined in OECD Guideline 476) only detects gene mutations, and the exogenous metabolic activation system

[18] 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).

[19] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352

	does not entirely mirror *in vivo* metabolism (i.e. the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[20] **Eye irritation**: The ICE test (OECD 438)[21] is only used to identify substances that cause serious eye damage (GHS Category 1) or to identify substances that do not require classification under EU-GHS (CLP). It cannot identify mild eye irritant (Category 2B) (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* gene mutation assay/ *in vitro* chromosome aberration 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	N	
Eye irritation	Y	*In vitro* test: Isolated Chicken Eye (ICE) test method (OECD Guideline 438):
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	N	
Bioaccumulation	N	

[20] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[21] https://www.oecd-ilibrary.org/docserver/9789264264618-en.pdf?expires=1624563757&id=id&accname=guest&checksum=E2F29943E63F6879BD21681BBE5A45D4

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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 CNFs (CAS #NA)

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	**CNFs**	**NA**	*H*	**M**	*L*	*L*	DG	**L**	**M**	**H**		*L*	**L**	*L*	**L**	*H*	**L**	*M*	*vH*	*vL*	*L*	**L**

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
CNFs	1

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

Table 6

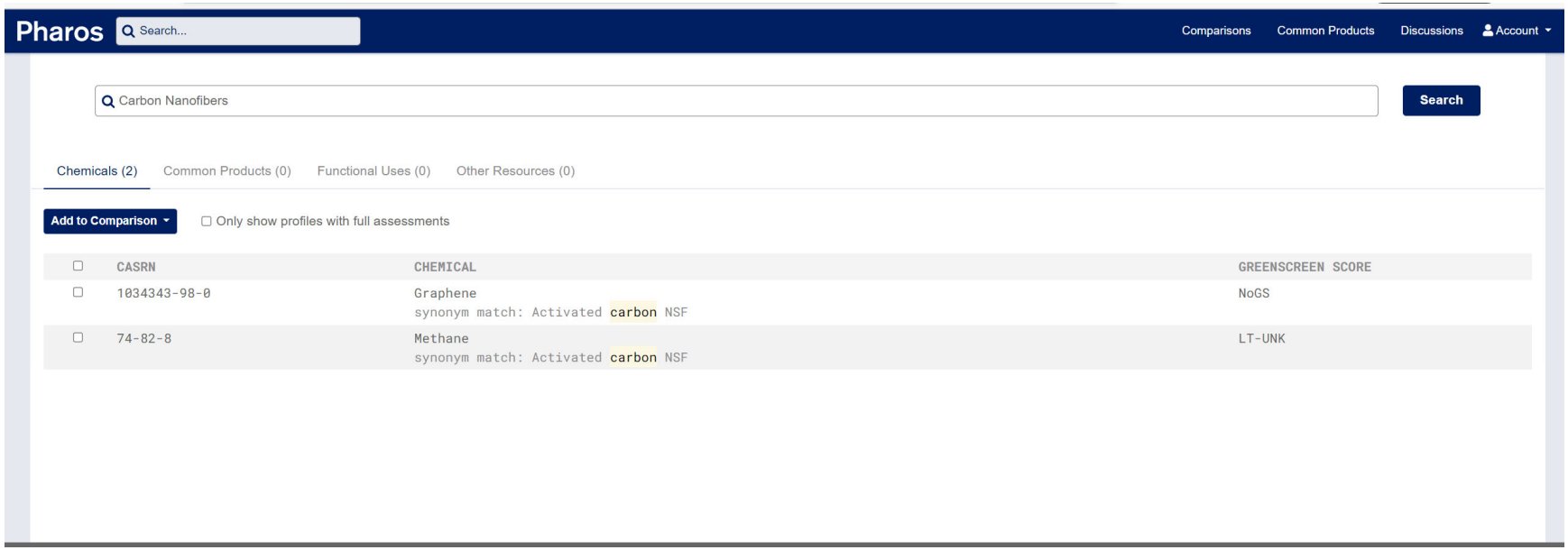
Chemical Name	Final GreenScreen® Benchmark Score
CNFs	1

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

Table 5: Data Gap Assessment Table

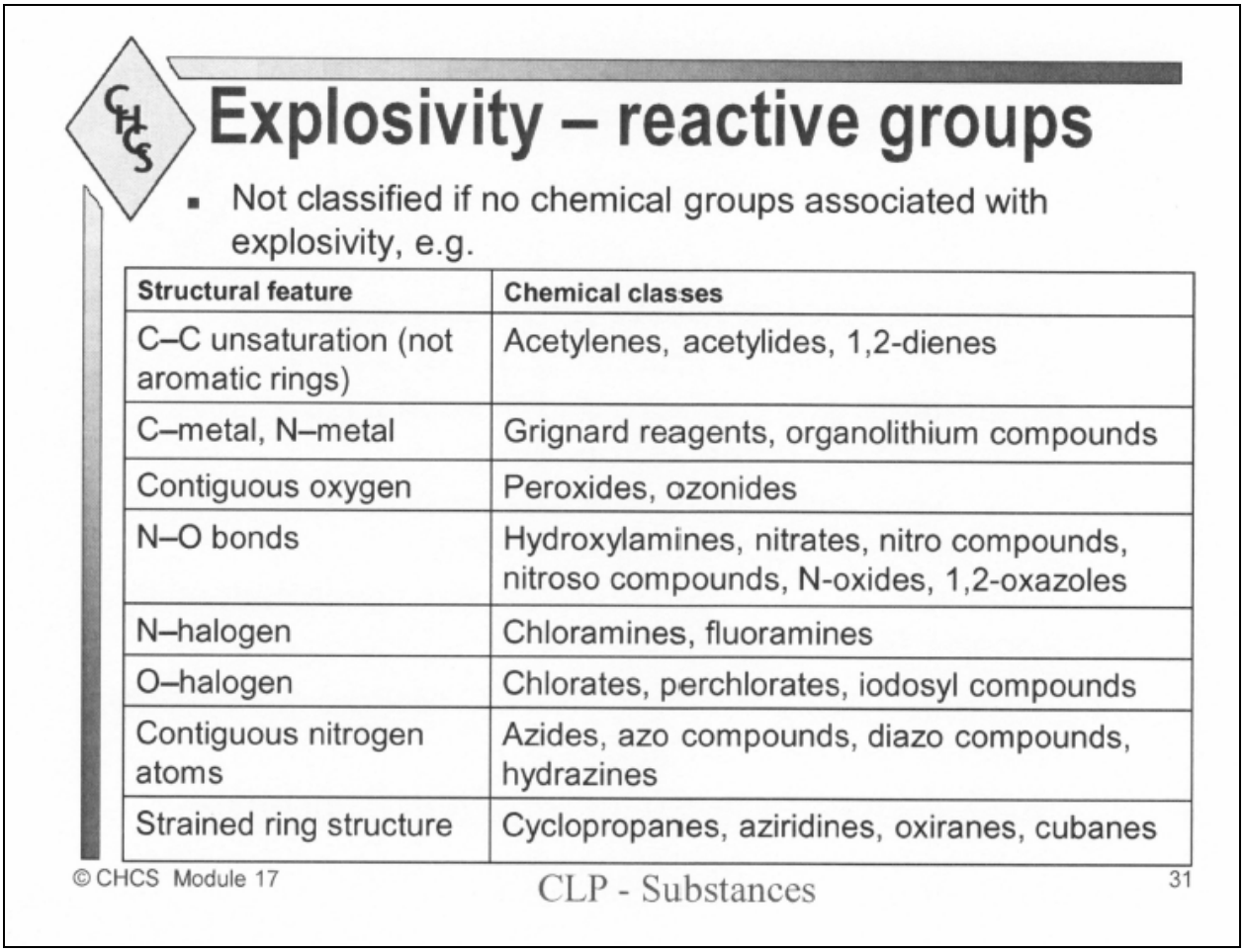
Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												1
2												
3												
4												

APPENDIX C: Pharos Output for CNFs (CAS #NA)



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

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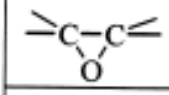
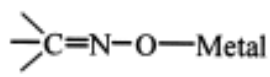
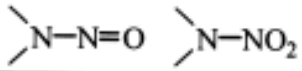
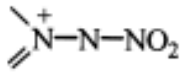
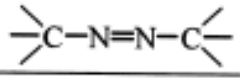
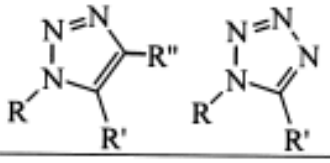
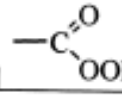
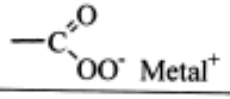
Explosivity – Full List

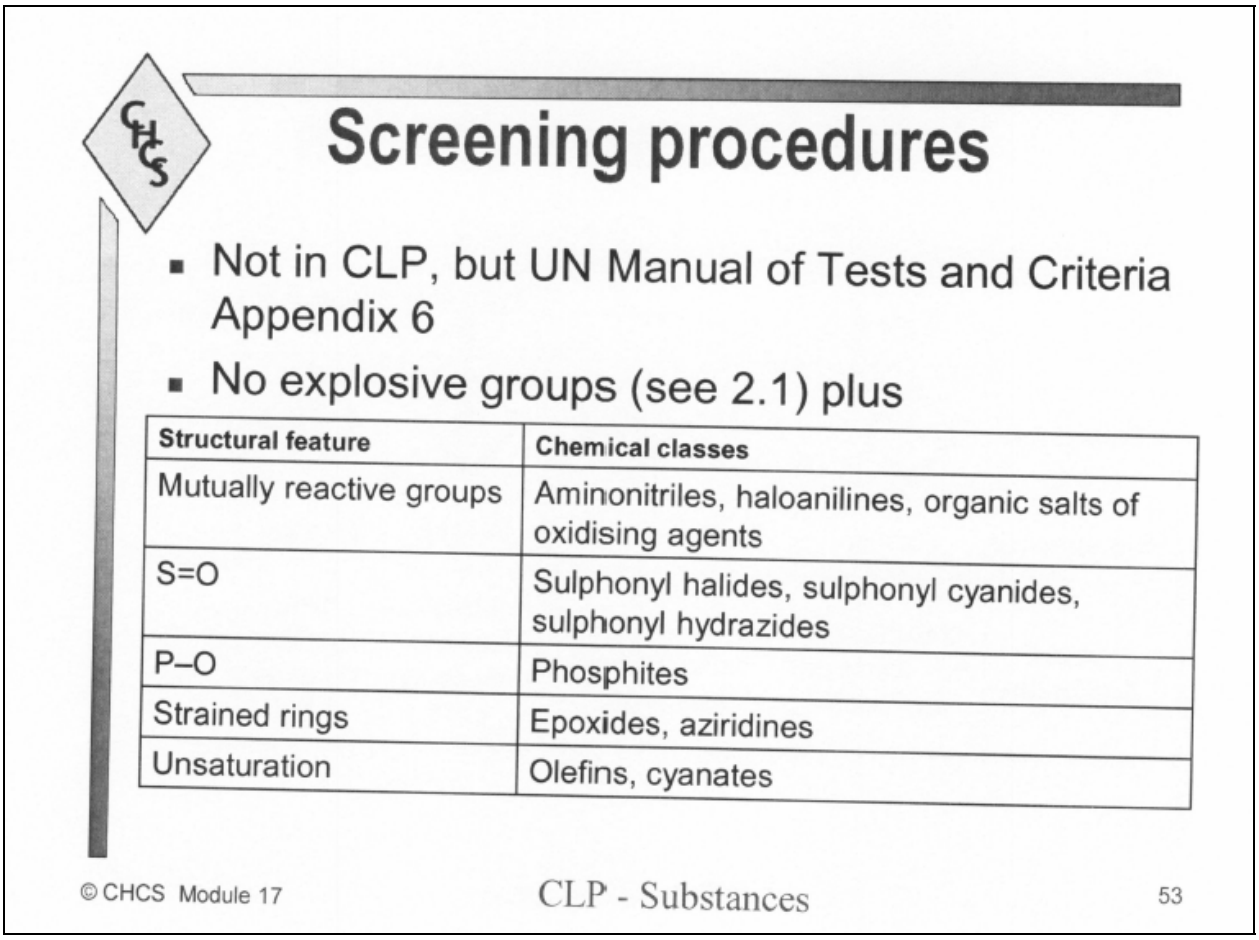
Table R.7.1-28 Chemical groups associated with explosive properties

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

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