NIA
Nanotechnology
Industries Association

April 5, 2022

**Heather Tenney**
Program Manager, Toxic Use Reduction Institute (TURI)
University of Massachusetts Lowell
126 John Street, Suite 14
Lowell, Massachusetts 01854-02866

(Sent by e-mail and Federal Express)

**RE: Carbon Nanotubes and Fibers Petition – SWCNT Literature Review Submission**

Dear Ms. Tenney,

On behalf of the Nanotechnologies Industry Association ("NIA"), I would like to respectfully submit the enclosed literature review regarding single-walled carbon nanotubes ("SWCNT"), as prepared by Dr. Julie Muller of ToxMinds for the TURI Scientific Advisory Board's consideration in its deliberations on whether or not to list SWCNTs on TURA's chemical list.

NIA is located in Brussels, Belgium and was founded in 2005 to be a responsible voice for nanotechnology value chains and the global commercial eco-system. As part of our activities, we promote the safe and reliable commercialization of nanoscale materials. We participate in the OECD's Working Party on Manufactured Nanomaterials as well as standardization committees such as ISO/TC 229 and CEN/TC 352. NIA also assists its members in complying with regulatory requirements, especially in the EU, related to the manufacturing and placing on the market of nanoscale materials. Further information on the organization and our activities can be found on www.nanotechia.org.

Our members have become aware of the Scientific Advisory Board's activities and asked us to prepare this submission, in order to assist in the Board's deliberations and ensure that it is aware of the most pertinent environmental, health, and safety studies regarding SWCNTs. Dr. Muller is a highly-respected regulatory toxicologist with an extensive background in carbon nanotubes. We hope that you will find her report helpful in your deliberations.

Although the literature review is marked "Confidential," TURI and the Scientific Advisory Board may use it for all purposes in their deliberations. Dr. Muller and I remain of course available for any questions.

Kind regards,

Chiara Venturini
Director General, NIA

CONFIDENTIAL

# Literature search report related to single-wall carbon nanotubes (SWCNT)

**11 March 2022**

**Prepared by:**

Dr Julie Muller

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# Contents

## 1. SUMMARY OF THE EXPERT CV

Dr. Julie Muller is a trained regulatory toxicologist. After a Ph.D. in biomedical and pharmaceutical sciences in the field of nano-toxicology (Subject: 'Experimental study of the respiratory toxicity of multi-walled carbon nanotubes;' in the Unit of Industrial Toxicology and Occupational Health at Université catholique de Louvain (UCL), Brussels, Belgium), Julie worked at Solvay S.A. as toxicologist in the department of Health, Safety and Environment in Belgium. After a few years, she moved to Nanocyl S.A. headquarter where she became health and safety manager in charge of the responsible care product stewardship programme of the company. She then acquired a new position as toxicology manager at Penman Consulting BVBA, a consultancy company dealing with the technical oversight of large REACH consortium and individual projects in the chemical sector. Since 2019, she is Senior Consultant Product Safety and Regulatory Affairs at ToxMinds BVBA. In this role, Julie is involved in all aspects of the human health evaluation of substances from a wide range of sectors, including chemicals, cosmetics, pharmaceutical impurities and metals.

**EDUCATIONAL BACKGROUND**

**2009** PhD in Biomedical and Pharmaceutical Sciences, orientation Toxicology, Genetic and Immunology - Université catholique de Louvain, Belgium

Subject: "Experimental study of the respiratory toxicity of multi-wall carbon nanotubes." in the Unit of Industrial Toxicology and Occupational Health directed by Professor D. Lison (Co-promoter Professor M. Kirsch- Volders)

**2006** Complementary master in Health Sciences - Université catholique de Louvain, Belgium

**2003** Master in Biomedical Sciences, orientation Toxicology- Université catholique de Louvain, Belgium

**CERTIFICATES AND PROFESSIONAL AFFILIATIONS**

Member of the Belgian (Eco)Toxicology Society (Beltox)

## 2. BACKGROUND

In October 2020, the Toxics Use Reduction Act (TURA) Program in Massachusetts (USA) received a petition to list carbon nanotubes (both single-walled and multi-walled) and carbon nanofibers. On that basis, the TURA Science Advisory Board (SAB) started reviewing these substances.

SAB's primary role is to consider petitions to add or delete chemicals from the TURA list and make recommendations to the Toxics Use Reduction Institute (TURI) accordingly. To conduct its assessment, SAB relies on scientific information to evaluate a range of standard health and environmental endpoints including: carcinogenicity, teratogenicity, reproductive dysfunction, neurotoxicity, genotoxicity, chronic or sub chronic health effects including asthma, sensitization, endocrine disruption, significant acute effects, toxicity, environmental toxicity and persistence, bioaccumulation in the environment, and other environmental effects such as ozone depletion, climate change, toxicity or breakdown of other products. The specific endpoints on which SAB will focus for the CNT evaluation are not yet clear hence, ToxMinds has restricted his search on standard toxicological endpoints.

Although SAB is currently focusing its assessment on multi-walled carbon nanotubes (MWCNT), its next focused will be to evaluate the potential impact of single-walled carbon nanotubes (SWCNT).

In support of this assessment, the following work was conducted:

- **Public literature and databases, desktop search**: Generation of a systematic literature search on specific standard toxicological endpoints. The period that was covered ranges from January 2010 to December 2021. The first focus was on carcinogenicity; reproductive and developmental toxicity; neurotoxicity; genotoxicity; acute, sub chronic or chronic toxicity and sensitization.
- **Collation of information in a data matrix**
- **Evaluation of quality/reliability of identified data/hits**, using the Klimisch method. The Klimisch score is a method of assessing the reliability of toxicological studies, mainly for regulatory purposes, that was proposed by H.J. Klimisch (1997). In this paper, the reliability is defined as 'the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings. It assigns studies to one of four categories as follows:
    - **1 – reliable without restriction**: "*studies or data [...] generated according to generally valid and/or internationally accepted testing guidelines*[a] *(preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline [...] or in which all parameters described are closely related/comparable to a guideline method.*"

[a] OECD Guidelines for the Testing of Chemicals, Section 4 : Health Effects | OECD Guidelines for the Testing of Chemicals | OECD iLibrary (oecd-ilibrary.org)
Hyperlink: https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

- **2 - reliable with restriction**: "*studies or data [...] (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable*."
- **3 - not reliable**: "*studies or data [...] in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g. unphysiological pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment*."
- **4 - not assignable**: "*studies or data [...] which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.)*."

The use of such scoring tools allows ranking/ organizing the information for further review. This implies focusing on the most relevant ones, taking into account the endpoint being measured or estimated. The evaluation of the reliability is performed considering certain formal criteria using international standards as references. The scoring of information should not exclude all unreliable data from further consideration by expert judgement because of possible pertinence of these data related to the evaluated endpoints.

Generally, only Klimisch scores of 1 or 2 can be used by themselves to cover an endpoint. However, Klimisch score 3 and 4 data can still be used as supporting studies or as part of a weight of evidence approach.

- **Development an overall summary table** with the key identified studies

## 3. LITERATURE SEARCH STRATEGY

In January 2022, a literature search on single-walled carbon nanotubes was carried out to identify the available literature from January 2010 to December 2021. The first focus was on acute toxicity, irritation / sensitization, repeated dose toxicity, mutagenicity / genotoxicity, carcinogenicity; reproductive / developmental toxicity and neurotoxicity. The following sites and portals were searched using the 'chemical name/structure' and specific keywords:

- Aggregate database: eChemPortal (OECD[a])
- Other regulatory and toxicity databases: Pubmed, Toxnet, Google search (including google scholar & google advanced), EPA comptox, and ToxPlanet, Europe PMC, U.S. EPA ACToR
- Scientific literature databases: PubMed, ToxNet, Science direct, Google Scholar. Search on Web of Knowledge (now Web of Science) and Scopus were not performed as it required subscription.

The literature search strategy follows standard practices and was performed: 1) first using a single concept search strategy in the specific databases (listed above) and 2) second using a combination of single concept and targeted search strategies.

The searches were carried out using the following search-strings:

- "Single Wall Carbon Nanotubes" OR "SWCNT"
- ("Single Wall Carbon Nanotubes" OR "SWCNT") AND ("rat" OR "rats" OR "mice" OR "mouse" OR "guinea pig" OR "rabbit" OR "rabbits") AND ("LD50" OR "LC50" OR "mortality" OR "death")
- ("single Wall Carbon Nanotubes" OR "SWCNT") AND ("rat" OR "rats" OR "mice" OR "mouse" OR "guinea pig" OR "rabbit" OR "rabbits" OR "monkey") AND ("reproduct" OR "reproductive" OR "reproduction" OR "development" OR "developmental" OR "malformation" OR "anomal" OR "fecund" OR "fertility" OR "foetus" OR "fetus" OR "estrogen" OR "oestrogen" OR "testosteron" OR "androgen" OR "Pup" OR "dam" OR "sperm" OR "hatch")
- ("Single Wall Carbon Nanotubes" OR "SWCNT") AND ("genotox" OR "genotoxicity" OR "mutation" OR "gene mutation" OR "chromosomal aberration" OR "mutagen" OR "mutagenicity")
- ("Single Wall Carbon Nanotubes" OR "SWCNT") AND ("rat" OR "rats" OR "mice" OR "mouse" OR "guinea pig" OR "rabbit" OR "rabbits") AND ("neurotoxicity")
- ("Single Wall Carbon Nanotubes"OR "SWCNT") AND ("mice" OR "mouse" OR "guinea pig") AND ("sensiti"OR "sensitization" OR "induction" OR "challenge" OR "rechallenge")
- ("Single Wall Carbon Nanotubes" OR "SWCNT") AND ("rat" OR "mice" OR "mouse" OR "guinea pig" OR "rabbit") AND ("carcinogen" OR "cancer" OR "tumour" OR "tumor" OR "metastatis")
- ("Single Wall Carbon Nanotubes" OR "SWCNT") AND ("rat" OR "rats" OR "mice" OR "mouse" OR "guinea pig" OR "rabbit" OR "rabbits") AND ("subchronic" OR "chronic" OR "subacute" OR "Toxicity" OR "toxic" OR "NOAEL" OR "NOEL" OR "BMDL" OR "LOEL" OR "LOAEL")

All identified hits were screened for relevancy in view of regulatory assessment, using adapted criteria's from (EFSA, 2011) (section 3.2. of the ECHA-EFSA ED guidance); (Klimisch *et al.*, 1997); (Vermeire *et al.*, 2013). All relevant hits containing the required data for the substances were tabulated in respective spreadsheets. This was followed by performing a reliability assessment of the identified relevant results as per the definition provided in section 3.2.1 of the ECHA-EFSA ED guidance.

This document reports the overall summary tables of key identified studies.

## 4. SUMMARY OF KEY TOXICOLOGICAL STUDIES IDENTIFIED

### 4.1. Acute toxicity

For the assessment of acute toxicity, the database comprises 1 study performed in 2012, as summarized in **Table 1**. The study used entangled SWCNT with diameter of 2 nm and specific surface area (SSA) was not specified. It was performed according to OECD Guidelines 423[a] in rats and meet the criteria for Klimisch score 1.

Under the conditions of this study, $LD_{50}$ was founded to be above the highest achievable tested dose of 50 mg/kg bw.

**Table 1.** Available studies: acute toxicity

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Matsumoto *et al.*, 2012<br>**KL1** | SWCNT[b]<br>(provided by AIST, Japan ; lot no. SW1859/SW1860/SW1865 produced by Nikkiso Co.) | SSA: N/A<br>Diameter: 2 nm<br>Length: N/A<br>Form of SWCNT: entangled | Route of administration: Oral gavage<br>Doses: 0 (control), 50 mg/kg bw/day<br>Vehicle: gum acacia<br>Duration of observation period following administration: 24 hours<br>Number of animals: 3 rats CRL:CD (SD)/group<br>Guideline: OECD Guideline 423[a]; GLP: Yes | LD50: >50 mg/kg bw |

[b] This study was also conducted with MWCNT.

### 4.2. Irritation / Sensitisation

For the assessment of irritation / sensitization, the database comprises 5 studies performed between 2011 and 2020, as summarized in **Table 2**. The studies used SWCNT with diameter ranging from 1.8 to 6 nm and specific surface areas (SSA) ranging from 700 to 878 m²/g. They were performed according to OECD[a] Guidelines 404, 405, 406, 442D or 442B in either rabbit, guinea pigs or mice and meet the criteria for Klimisch score 2.

Under the conditions of these studies, SWCNT was not founded to be corrosive, irritant or a skin sensitiser.

**Table 2.** Available studies: irritation / sensitisation

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| **a) Eye irritation** | | | | |
| Ema *et al.*, 2011<br>**KL2** | SWCNT[c] (Nikkiso-SWCNTs/N-SWCNTs, Super-growth SWCNTs/SG-SWCNTs) | **N-SWCNT**<br>SSA: 878 $m^2/g$<br>Diameter: 1.8 nm<br>Length: N/A<br>Form of SWCNT: Not specified<br>**SG-SWCNTs**<br>SSA: 1064 $m^2/g$<br>Diameter: 3.0 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of study/test substance application: *In vivo* eye irritation / instillation into the conjunctival sac 72 hours observation period<br>Doses: 0.1% N-SWCNTs, 0.5% SG-SWCNTs in vehicle<br>Vehicle: olive oil<br>Number of animals: 3 male Kbl:NZW rabbits / dose<br>Guideline: OECD Guideline 405[a]; GLP: no | Not irritant<br>Ocular responses, such as corneal opacity, conjunctival redness, abnormality of the iris, and chemosis, were not detected in rabbits instilled with N-SWCNTs, SG-SWCNTs. |
| **b) Skin irritation** | | | | |
| Ema *et al.*, 2011<br>**KL2** | SWCNT[d] (Nikkiso-SWCNTs/N-SWCNTs, Super-growth SWCNTs/SG-SWCNTs) | **N-SWCNT**<br>SSA: 878 $m^2/g$<br>Diameter: 1.8 nm<br>Length: N/A<br>Form of SWCNT: Not specified<br>**SG-SWCNTs**<br>SSA: 1064 $m^2/g$<br>Diameter: 3.0 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of study/test substance application: *In vivo* skin irritation / patch on intact shave skin 4 hours exposure, 72 hours observation period<br>Coverage: semi occlusive<br>Doses: 1% in vehicle<br>Vehicle: olive oil<br>Number of animals: 3 male Kbl:NZW rabbits /dose<br>Guideline: OECD Guideline 404[a]; GLP: no | Not irritant<br>No dermal responses, including erythema/eschar or edema, were found in rabbits treated with N-SWCNTs, SG-SWCNTs. |
| **c) Skin sensitization** | | | | |

[c] This study was also conducted with MWCNT.
[d] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Ema *et al.*, 2011<br>**KL2** | SWCNT[e]<br>(Nikkiso-SWCNTs/N-SWCNTs, Super-growth SWCNTs/SG-SWCNTs) | **N-SWCNT**<br>SSA: 878 $m^2/g$<br>Diameter: 1.8 nm<br>Length: N/A<br>Form of SWCNT: Not specified<br>**SG-SWCNTs**<br>SSA: 1064 $m^2/g$<br>Diameter: 3.0 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of study/test substance application: *In vivo* skin sensitisation (Guinea Pig Maximisation Test (GPMT))<br>Coverage: occlusive<br>Doses: 1% in vehicle<br>Vehicle: olive oil<br>Positive control: 1-chloro 2,4-dinitrobenzene (DNCB)<br>Number of animals: 10 or 20 male Slc:Hartley guinea pigs /group<br>Guideline: OECD Guideline 406[a]; GLP: no | Non sensitizer<br>No erythema or edema was observed after the challenge with SWCNTs |
| Kim *et al.*, 2020<br>**KL2** | SWCNT<br>(Sigma Aldrich, No 704121) | SSA: ≥700 $m^2/g$<br>Diameter: 5.97 ± 1.48 nm<br>Length: 1 µm<br>Form of SWCNT: entangled | Type of study: *In vitro* skin sensitisation (ARE-Nrf2 luciferase LuSens)<br>Doses: 0.05 - 1000 µg/mL<br>Solvent control: DMEM + 1% FBS<br>Positive control: cinnamic aldehyde, 4–64 µM<br>Guideline: OECD Guideline 442D[a]; GLP: N/A | Non sensitiser<br>SWCNT did not induce the activity of the luciferase reporter in contrast to the positive control. |
| Kim *et al.*, 2020<br>**KL2** | SWCNT<br>(Sigma Aldrich, No 704121) | SSA: ≥700 $m^2/g$<br>Diameter: 5.97 ± 1.48 nm<br>Length: 1 µm<br>Form of SWCNT: entangled | Type of study: *In vivo* skin sensitisation (LLNA Assay) - dorsal skin ear application<br>Vehicle: distilled water<br>Doses: 0, 25, 50, 100 %<br>Positive control: 25% hexyl cinnamic aldehyde in AOO<br>Number of animals: 4 female BALB/C mice /group<br>Guideline: OECD Guideline 442B[a]; GLP: N/A | Non sensitiser<br>No significant effects were observed at any tested concentration. |

[e] This study was also conducted with MWCNT.

### 4.3. Repeated dose toxicity

For the assessment of repeated dose toxicity, the database comprises 2 sub-acute and 2 sub-chronic studies performed between 2011 and 2012, as summarized in **Table 3**. The studies used SWCNT with diameter ranging from 1 to 3 nm and specific surface areas (SSA) ranging from 1000 to 1064 $m^2/g$. They were performed according to OECD Guidelines 407[a] (or similar) in rats after inhalation, oral (gavage) or intra-tracheal exposure and meet the criteria for Klimisch score 1 or 2.

Under the conditions of the subacute toxicity studies, no toxicological effects were founded up to the highest dose. Results of the subchronic toxicity studies showed an inflammatory response restricted to the lung and lung associated lymph nodes. Fibrosis, atypical lesion, or tumor-related findings were not observed.

**Table 3.** Available studies: repeated dose toxicity

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| **a) Subacute toxicity** | | | | |
| Matsumoto *et al.*, 2012<br>**KL1** | SWCNT[f]<br>(provided by AIST, Japan ; lot no. SW1859/SW1860/SW1865 produced by Nikkiso Co.) | SSA: N/A<br>Diameter: 2 nm<br>Length: N/A<br>Form of SWCNT: entangled | Type of study: sub-acute toxicity<br>Route of administration: oral gavage<br>Doses: 0, 0.125, 1.25 or 12.5 mg/kg bw/day<br>Vehicle: gum acacia<br>Duration of treatment/exposure: 28 days<br>Recovery period: 14 days<br>Number of animals: 5 or 10 rats CRL:CD (SD)/ sex / group<br>Guideline: OECD Guideline 407[a]; GLP: yes | NOAEL: 12.5 mg/kg bw/day No toxicological effects were founded up to the highest dose. |

[f] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Morimoto *et al.*, 2012 **KL2** | SWCNT (AIST, Japan) | SSA: 1064 ± 37 $m^2/g$ Diameter: 3.0 ± 1.1 nm Bundle length: 0.2 µm Form of SWCNT: entangled | Type of study: sub-acute toxicity Route of administration: inhalation (whole body exposure) Doses: 0, 0.03, 0.13 $mg/m^3$ Vehicle: distilled water + Tween 80 Duration of treatment/exposure: 6 h/day, 5 days/week, for 4 weeks Recovery period: 3d, 1 and 3 months Number of animals: 10 male Wistar rats / dose Guideline: N/A; GLP: no | None of the tested concentrations of SWCNT induced a modification of the inflammatory parameters investigated. SWCNT did not induce neutrophil inflammation in the lung |
| **b) Subchronic toxicity** | | | | |
| Kobayashi *et al.*, 2011 **KL2** | SWNCT (AIST, Japan) | SSA 1000: $m^2/g$ Diameter: 1-3 nm Bundle length: 0.32 µm Form of SWCNT: N/A | Type of study: subchronic toxicity Route of administration: intratracheal instillation Doses:0, 0.2 and 2 mg/kg Vehicle: Tween 80 and PBS dissolved in Milli-Q water Duration of treatment/exposure: 3 months Number of animals: 5 rats CRL: CD(SD) male/ group Guideline: N/A; GLP: no | Dose related inflammatory response was observed only in lungs and lung-associated lymph nodes. Progressive lung tissue thickening was observed in the highest dose (2mg/kg) group. Fibrosis, atypical lesion, or tumor-related findings were not observed. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Kobayashi *et al.*, 2011<br>**KL2** | SWNCT<br>(AIST, Japan) | SSA 1000: $m^2/g$<br>Diameter: 1-3 nm<br>Bundle length: 0.32 µm<br>Form of SWCNT: N/A | Type of study: subchronic toxicity<br>Route of administration: intratracheal<br>instillation<br>Doses: 0, 0.04, 0.2, 1.0 mg/kg<br>Vehicle: Tween 80 and PBS dissolved in Milli-Q water<br>Duration of treatment/exposure: 6 months<br>Number of animals: 6 rats CRL: CD(SD) male/ group<br>Guideline: N/A; GLP: no | Dose related inflammatory response was observed only in lungs and lung-associated lymph nodes. Fibrosis, atypical lesion, or tumor-related findings were not observed in all groups up to 6 months after instillation. SWCNTs did not induce pulmonary inflammation<br>at 0.04mg/kg of pulmonary deposition. |

### 4.4. Reproductive / developmental toxicity

For the assessment of reproductive / developmental toxicity, the database comprises 10 studies performed between 2007 and 2020, as summarized in **Table 4**. The studies used SWCNT with diameter ranging from 1 to 11 nm. They were performed either *in vitro/ex vivo* on embryonic stem cells or *in vivo* in mice and meet the criteria for Klimisch score 2, 3 or 4.

The available identified data provide initial information that several types of SWNT possess the potential for reproductive and developmental toxicity. The database is still very limited, especially when taking into account the many different particle types and their potential surface modifications (e.g., functionalized vs non functionalized SWCNT). It follows that the insufficient database also hinders hazard identification and risk assessment on reproductive and developmental toxicity of nanomaterials.

***Of note***: In addition to the dataset identified via the present literature search, a company owned OECD 422[a] study (data owner is willing to share these results with the TURA SAB) is reported here below:

A reproductive and developmental screening study was conducted with SWCNT (pristine – no surface treatment - Tuball™, see additional characterization information in **Annex 1**), according to OECD Guideline 422[a], in compliance with GLP. SWCNTs were administered orally to rats via the diet at dose levels of 100, 300 or 1000 mg/kg bw/day (corresponding to mean actual intake of 102, 305 and 1026 mg/kg bw/day for the male and to 117, 341 and 1195 mg/kg bw/day for the female) prior to, during and after mating to generate limited information concerning the effects of the test item on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition.

The animals were checked at least twice daily during the dosing period for mortality and morbidity and once daily for clinical signs. Detailed clinical observations were performed once a week. Body weight and food consumption were recorded once a week during premating and mating periods (food consumption not during mating), and during gestation on Days 0, 7, 14 and 20 p.c. and lactation on Days 1, 4, 8 and 13 p.p. The animals were paired for mating after 2 weeks of treatment. The total litter sizes and the sex of each pup were recorded after birth. The pups were observed daily for clinical signs, abnormal behaviour and external abnormalities and weighed. At the end of the treatment period, Functional Observation Battery, motor activity and laboratory investigations (haematology, coagulation, clinical biochemistry, thyroid hormone) were carried out on five males and five females. At the time of sacrifice or death, the animals were weighted and examined macroscopically. The reproductive organs and all organs of the adult animals showing macroscopic lesions were preserved. Histopathological examination was performed for selected organs from the animals of the low dose group and the high dose group and for all organs of the adult animals showing macroscopic lesions. The pups were submitted for a macroscopic post-mortem examination.

None of the animals died prematurely. No changes were noted in behaviour, the external appearance and the consistency of the faeces. Black discoloured faeces that were noted for all males and females at the high dose group were due to the high concentration of the administered test item (black powder) and not of toxicological relevance. No influence was noted for the male and female animals on body weight, food consumption, on the results of the neurological screening, the haematological and biochemical parameters and the T4 level of the male animals. No test item-related changes were noted during the macroscopic and the microscopic examinations and for the relative and absolute organ weights. The mean actual intake of the test item via the diet over the whole study period was 102, 305 and 1026 mg Tuball™/kg bw/day for the male and 117, 341 and 1195 mg Tuball™/kg bw/day for the female animals. Hence, the actual values were in the range of the nominal values with 100, 300 or 1000 mg Tuball™/kg bw/day. No influence was noted on the fertility index, the gestation index, the duration of the pre-coital time interval and the gestation period. No test item-related effect was

noted on the prenatal development of the pups (birth and live birth index, percentage of post implantation loss)

Furthermore, no test item-related effect was noted on the postnatal development of the pups with regarding to the viability indices (pre- and post-cull), the pup body weight, the ano-genital distance, the nipple retention, the T4 level and the histopathological examination of the pup thyroids. No variations or malformations were noted during the macroscopic external examination at necropsy.

Under the study conditions, the no-observed-adverse-effect levels (NOAEL) for parental systemic toxicity, for reproductive performance and for toxic effects on progeny was considered to be higher than the highest tested dose >1000 mg/kg bw/day (Leuschner, 2020).

**Table 4.** Available studies: reproductive / developmental toxicity

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| ***a) In vitro/ex vivo* embryonic developmental toxicity** | | | | |
| Roman *et al.*, 2013<br>**KL3** | SWCNT<br>(Sigma-Aldrich, Inc., Oakville, Canada) | SSA: N/A<br>Diameter: 11 nm<br>Length: 0.5-100 µm<br>Form of SWCNT: N/A | Type of study: embryonic stem cell test in chicken<br>Route of administration: embryo incubation<br>Doses: 25 µg/embryo<br>Vehicle: PBS<br>Duration of treatment/exposure: day 3 until days 11-14<br>Number of animals: 75 treated White Leghorn chicken embryos and 18 controls White Leghorn chicken embryos<br>Guideline: N/A; GLP: N/A | A total of 80% of the embryos injected with SWCNTs died before incubation day 12. No embryonic anomalies were detected, but exposure inhibited growth and angiogenesis in several organs and deregulated expression of genes involved in cell proliferation, apoptosis, survival, cell cycle, and angiogenesis. This suggests that SWCNTs could have a toxic effect on the normal development of the embryo. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Ema *et al.*, 2016<br>**KL4**<br>(Source: Pietroiusti *et al.*, 2011) | SWCNT<br>(Cheap Tubes inc.; Pristine (p)-SWCNT, Oxidised (o)-SWCNT and ultra oxide (uo)-SWCNT) | **-(p)-SWCNT**<br>SSA: N/A<br>Diameter: 2.37+/-0.4nm<br>Length: 0.85+/-0.42<br>Form of SWCNT: Pristine<br>**- (o)-SWCNT**<br>SSA: N/A<br>Diameter: 1.58+/-0.2 nm<br>Length: 0.76 +/-0.7<br>Form of SWCNT: Surface treated<br>**- (uo)-SWCNT**<br>SSA: N/A<br>Diameter: 1.8 +/-0.4 nm<br>Length: 0.37 +/-0.17<br>Form of SWCNT: Surface treated | Type of study: embryonic stem cell test in mice<br>Route of administration: embryo incubation<br>Doses: 0.1, 1, 2, 6, 10mg/mL<br>Vehicle: DMEM+BSA<br>Duration of treatment/exposure: 10 days<br>Guideline: N/A; GLP: N/A | Decreased cell viability (from 1 mg/mL) and impairment of the differentiation into contracting embryoid bodies (EBs) (starting at 6 mg/mL) were observed. The three types of SWCNTs were found to be strong embryotoxic. |
| Ema *et al.*, 2016<br>**KL4**<br>(Source: Cheng *et al.*, 2007) | SWCNT<br>(Sigma Aldrich) | SSA: N/A<br>Diameter: 11 nm<br>Length: 0.5-100 µm<br>Form of SWCNT: N/A | Type of study: embryonic stem cell test in zebrafish<br>Route of administration: embryo incubation<br>Doses: 20, 40, 60, 120, 240, 360 µg/mL<br>Vehicle: Water<br>Duration of treatment/exposure: 4-96 hpf<br>Guideline: N/A; GLP: N/A | Most of the agglomerates were microscale and adhered to the outer layer of the chorion, too large to enter. SWCNTs transiently delayed embryo hatching, but did not influence hatching success, survival rate, or morphological, molecular, or cellular development of embryos. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Ema *et al.*, 2016<br>**KL4**<br>(Source: Philbrook *et al.*, 2011) | SWCNT<br>(MK Nano, Canada - functionalised (OH)-SWCNT) | SSA: N/A<br>Diameter: 1-2 nm<br>Length: 5-30 µm<br>Form of SWCNT: Surface treated | Type of study: embryonic stem cell test in drosophila melanogaster<br>Route of administration: embryo incubation<br>Doses: 0.005, 0.01, 0.05, 0.1, 0.5%<br>Vehicle: PBS<br>Duration of treatment/exposure: Day 3<br>Guideline: N/A; GLP: N/A | OH-SWCNTs did not influence fecundity, fertility, or development until the adult stage. |
| ***b) In vivo* toxicity to reproduction** | | | | |
| Ivani *et al.*, 2016<br>**KL2** | SWCNT<br>(Research Institute of Petroleum Industry, Tehran, Iran) | SSA: N/A<br>Diameter: 1-2 nm<br>Length: 10 µm<br>Form of SWCNT: N/A | Type of study: reproductive toxicity (effect of prenatal exposure)<br>Route of administration: intraperitoneal injection<br>Doses: 1 and 10 mg/kg bw<br>Vehicle: PBS<br>Duration of treatment/exposure: GDs 0 and 3<br>Number of animals: 10 pregnant females NMRI mice/ group<br>Guideline: N/A; GLP: N/A | A decrease in litter size on postnatal day 2 was observed in the group treated with 10 mg/kg b.w. of SWCNTs whereas no significant differences between groups were observed in any other parameters. The behavioral development of pups did not show significant differences during growth except for the surface righting reflex, which showed significant delay compared to control in the group treated with 1 mg/kg b.w. SWCNTs. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Farshad *et al.*, 2020 **KL3** | SWCNT[g] (US Research Nanomaterials Inc., Houston USA; Stock #: US4112, CAS: 99685-96-8) | SSA: N/A<br>Diameter: 1-2 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of study: reproductive toxicity<br>Route of administration: oral gavage<br>Doses: 0, 10, and 50 mg/kg/day<br>Vehicle: distilled water<br>Duration of treatment/exposure: 5 weeks<br>Number of animals: 8 BALB/c mice male / group<br>Guideline: N/A; GLP: N/A | SWCNT impacted epididymal sperm characteristics (decrease sperm viability / motility, sperm count // increase of percent abnormal sperm, testicular and sperm TBARS contents).<br>Significant histopathological and stereological alterations in the testis occurred in the groups challenged with SWCNTs.<br>Current findings indicated that oxidative stress and mitochondrial dysfunctionality seem to be the primary mechanisms in inducing CNT toxicity in the reproductive system. |
| ***c)*** **Developmental toxicity / teratogenicity** | | | | |

[g] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Ema *et al.*, 2016<br>**KL4**<br>(Source: Pietroiusti *et al.*, 2011) | SWCNT<br>(Cheap Tubes inc.; Pristine (p)-SWCNT, Oxidised (o)-SWCNT and ultra oxide (uo)-SWCNT) | **-(p)-SWCNT**<br>SSA: N/A<br>Diameter: 2.37+/-0.4nm<br>Length: 0.85+/-0.42<br>Form of SWCNT: Pristine<br>**- (o)-SWCNT**<br>SSA: N/A<br>Diameter: 1.58+/-0.2 nm<br>Length: 0.76 +/-0.7<br>Form of SWCNT: Surface treated<br>**- (uo)-SWCNT**<br>SSA: N/A<br>Diameter: 1.8 +/-0.4 nm<br>Length: 0.37 +/-0.17<br>Form of SWCNT: Surface treated | Type of study: developmental toxicity<br>Route of administration: intravenous injection<br>Doses: 3 µg/mice of (p)-SWCNT, 30 µg/ mice of o-SWCNT, 0.3 µg and higher of (u)-SWCNT at GD 5.5<br>Vehicle: DMEM-BSA<br>Duration of test: GD 15.5<br>Number of animals: 16-23 CD1 mice /group<br>Guideline: N/A; GLP: N/A | A high percentage of early miscarriages and fetal malformations was observed in females exposed to o-SWCNTs, while lower percentages were found in animals exposed to the p-SWCNTs. Deformities in the abdominal wall and head and limb hypoplasia were observed. The placentas of malformed fetuses were small and vascular-damaged in the labyrinth layer. Following an injection of uo-SWCNTs, the levels of ROS were higher in malformed fetuses and their placentas. The incidence of abortion was increased in mice injected with p-, o-, and uo-SWCNTs at 30 µg/mice on GD 5.5. Overall, data suggests that SWCNTs may act as embryotoxic agents in mammals. |
| Ema *et al.*, 2016<br>**KL4**<br>(Source: Philbrook *et al.*, 2011) | SWCNT<br>(MK Nano, Canada - functionalised (OH)-SWCNT) | SSA: N/A<br>Diameter: 1-2 nm<br>Length: 5-30 µm<br>Form of SWCNT: Surface treated | Type of study: developmental toxicity<br>Route of administration: oral gavage<br>Doses: 10 and 100 mg/kg<br>Vehicle: 0.5% (w/v) tragacanth gum solution in DI water<br>Duration of treatment/exposure: Single exposure / GD 9<br>Duration of test: until GD 19<br>Number of animals: 10-12 female CD1 Mice/group<br>Guideline: N/A; GLP: N/A | Administration of functionalised SWCNT to pregnant CD-1 dams during organogenesis significantly increased the number of resorptions and resulted in fetal morphological and skeletal abnormalities after the administration of 10 mg/kg, but not 100 mg/kg. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Ema *et al.*, 2016<br>**KL4**<br>(Source: Campagnolo *et al.*, 2013) | SWCNT<br>(Carbon solution Inc., functionalised PEG-SWCNT) | SSA: N/A<br>Diameter: N/A<br>Length: 86 m<br>Form of SWCNT: Surface treated | Type of study: developmental toxicity (placenta transfer)<br>Route of administration: intravenous injection<br>Doses: 0.1-30 µg/mice at GD 5.5 or 10 µg/mice /day at GD 5.5, 8.5 and 11.5<br>Vehicle: PBS<br>Duration of treatment/exposure: GD 5.5, 8.5 and 11.5<br>Number of animals: 5-18 CD1 female mice / group<br>Guideline: N/A; GLP: N/A | Functionalised SWCNTs may cause occasional teratogenic effects in mice beyond a threshold dose.<br>No adverse effects were observed both on embryos and dams up to the dose of 10 µg/mouse. At the dose of 30 µg/mouse, occasional teratogenic effects, associated with placental damage, were detected both when administered as a single bolus or as multiple doses. Hepatic damage in dams was seen only in the multiple exposure group. |
| Ema *et al.*, 2016<br>**KL4**<br>(Source: Huang *et al.*, 2014) | SWCNT[h]<br>(Cheap tube Inc.; functionalised PL-PEG-NH4-SWCNT) | SSA: N/A<br>Diameter: 1-2 nm<br>Length: 0.5-2 µm<br>Form of SWCNT: Surface treated | Type of study: developmental toxicity<br>Route of administration: intravenously injection<br>Doses: 2 mg/kg/day on GD 10.5, 12.5 and 15.5<br>Vehicle: water<br>Duration of test: 30 days<br>Number of animals:4-6 p53+/- mice /group<br>Guideline: N/A; GLP: N/A | No effect on maternal or fetal body weight, or incidence of fetal deformities. No significant changes were observed in dams or fetuses. Radioactivity and TEM revealed distribution of SWCNTs to fetal liver and placenta, but not brain, after injection on GD 15.5. |

[h] This study was also conducted with MWCNT.

### 4.5. Mutagenicity / Genotoxicity

For the assessment of mutagenicity / genotoxicity, the database comprises 29 *in vitro* studies (4 gene mutation assays in bacteria / 23 cytogenicity or MN formation assays / 2 gene mutation assays in mammalian cells) and 9 *in vivo* mutagenicity studies performed between 2007 and 2015, as summarized in **Table 5**. The studies used SWCNT with specific surface areas (SSA) ranging from 293 to 1128 $m^2/g$ and diameter ranging from 0.7 to 4.4 nm. They were performed according to OECD Guidelines 471, 473, 474, 487, 489[a] (or similar) in various cell lines and species and meet the criteria for Klimisch score 1, 2, 3 or 4.

Under the conditions of these studies, various controversial results were observed after exposure to SWCNT. Some studies showed that SWCNTs may induce genetic lesions such as DNA breaks, oxidised DNA bases, mutations, micronucleus formation, and chromosomal aberrations mainly mediated via the production of reactive oxygen species (ROS) while in other *in vivo* and *in vitro* reports, SWCNTs appear not to pose a genotoxic risk. Even if these findings could support a mechanism relevant with regards to genotoxicity and potentially to carcinogenicity, the heterogeneity in the types of carbon nanotubes evaluated in experimental studies, as well as the mechanistic data gaps regarding their potential carcinogenicity, precluded a generalisation to all types of CNTs.

***Of note***: After CNT synthesis, the rough material contains amorphous carbon and metal catalyst residues (usually Al, Fe, Co, Ni or Mo), which might be present at the surface of the CNT, as well as metallic clusters entrapped within the CNT. Post-synthesis treatment can modify CNT characteristics, including purity, adsorptive nature, aspect ratio, surface reactivity, hydrophilic properties and surface functionalization. Therefore, three main sources of variation can contribute to explain the varying profiles of CNT and so, the diversity of results found in the scientific literature:

1) *size and shape* (e.g. length, flexibility, aggregation/agglomeration state),

2) *presence of contaminants* (e.g. catalytic residues, PAH) and

3) *surface chemistry* (e.g. surface defect; functionalization)

Several physico-chemical features of CNT such as metallic contaminants, size, degree of oxidation or hydrophilic properties have been found to modulate their toxicity (Bottini *et al.* 2006; Kagan *et al.* 2006; Magrez *et al.* 2006; Sato *et al.* 2005; Shvedova *et al.* 2003, Donaldson *et al.*, 2006; Johnston *et al.*, 2010). CNTs with high aspect ratios and long, thin, rigid, and biopersistent properties may induce pulmonary responses similar to those observed with asbestos (Poland *et al.*, 2008; Braackhuis *et al*., 2014). Length is one of the critical factors underlying the potential toxicity of fibrous nanomaterials (Fujita *et al*., 2015). Therefore, fiber length is accepted as the major contributing factor in fiber pathogenicity (Schinwald *et al*, 2012). It has been reported that long fibers are cleared slowly from the lung, and therefore, are retained in the lung for long periods. This causes persistent lung burden, as the fibers are not easily engulfed by macrophages. This further leads to frustrated phagocytosis (Donaldson *et al*., 2006, 2010). By opposition, curled and tangled CNTs, do not induce pulmonary response as asbestos does.

Overall, it is still difficult to make a global generalization about the potential impact of CNT on human health, especially when considering the diverging data from *in vitro* and *in vivo* studies (Bottini *et al.* 2006; Cui *et al.* 2005; Davoren *et al.* 2007; Monteiro-Riviere *et al.* 2005; Pulskamp *et al.* 2007; Shvedova *et al.* 2003; Worle-Knirsch *et al.* 2006). Depending on the mode of production and post-synthesis modifications and because CNT are manufactured by several different companies, they may contain different amounts / types of impurities, chemical functions on their surface, … Homogeneity between batches from the same source can also vary substantially. A wide variety of CNT are thus produced, presenting a large variety of physical and chemical properties that may be the cause of the discrepancies reported among some of the initial toxicological studies.

Although a number of studies on SWCNT have recently been published, clear characterisation of the test materials including sample preparation is often missing, although this is essential for ensuring reproducibility and reliability of a study. Importantly, modifications of some specific characteristics of the SWCNT tested such as variations in tube diameter or length distribution might give rise to differences in the (eco-)toxicological profile. Effects of different SWCNT seem to depend on the form (length) and physico-chemical properties (metal content, aggregation/agglomeration, surface chemistry, and functionalisation). Thus, for the time being, a case-by-case approach supplemented by the weight of evidence from literature data is appropriate to generate a first preliminary robust assessment of a product of interest.

**Table 5.** Available studies: mutagenicity / genotoxicity

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| ***a) In vitro gene mutation in bacteria*** | | | | |
| Naya *et al.*, 2011<br>**KL1** | SWCNT<br>(AIST, Japan) | SSA: 1064 ± 37 $m^2/g$<br>Diameter: 3.0 ± 1.1 nm<br>Length: 1200 nm<br>Form of SWCNT: N/A | Type of assay: Ames test<br>Strain: *S. typhimurium* TA97, TA98, TA100, TA1535 and *E. coli* WP2 *uvr*A/pKM101<br>Metabolic activation: w and w/o S9<br>Concentrations: 12.5, 25, 50, 100, 200 and 500 µg/plate<br>Vehicle: Na CMC-Tween 80<br>Duration of exposure: 48h<br>Guideline: OECD 471[a]; GLP: N/A | SWCNTs did not increase the mean number of revertants of any *S. typhimurium* strain or the *E. coli* strain per plate at any concentration, w and w/o metabolic activation, in comparison to the spontaneous reversion rate in the negative control. There was no evidence of cytotoxicity under any of the test conditions. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Ema *et al.*, 2013<br>**KL1** | SWCNT<br>(Nikkiso Co. Ltd. Tokyo, Japan; N-SWCNT) | SSA: 878 $m^2/g$<br>Diameter: 1.8 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: Ames test<br>Strain: *S. typhimurium* TA98, TA100, TA1535, TA1357 and *E. coli* WP2uvrA<br>Metabolic activation: w and w/o S9<br>Concentrations: 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg per plate<br>Vehicle: Na CMC<br>Duration of exposure: 48h<br>Guideline: OECD 471[a]; GLP: N/A | SWCNTs had no mutagenic effects in any strains of *S. typhimurium* or E. coli WP2uvrA w and w/o metabolic activation. |
| Kim *et al.*, 2015<br>**KL1** | SWCNT<br>(Hanwha Nanotech, Incheon, Korea) | SSA: N/A<br>Diameter: 1-1.2 nm<br>Length: 20 µm<br>Form of SWCNT: N/A | Type of assay: Ames test<br>Strain: *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA<br>Metabolic activation: w and w/o S9<br>Concentrations: 31.3, 62.5, 125, 250, 500 µg/plate<br>Vehicle: F12 medium<br>Duration of exposure: 44-48h<br>Guideline: OECD 471[a]; GLP: N/A | The number of revertant colonies did not significantly increase in any of the treatment concentrations of the Salmonella and *E. coli* strains, w or w/o metabolic activation. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013<br>**KL4**<br>(Source: Kisin *et al.*, 2007; KL3) | SWCNT<br>(CNI, Inc., Houston, TX) | SSA: 1040 $m^2/g$<br>Diameter: N/A<br>Length: 1–3 µm<br>Form of SWCNT: N/A | Type of assay: Ames test<br>Strain: *S. typhimurium* YG1024 and YG1029<br>Metabolic activation: w/o S9<br>Concentrations: 60, 120 and 240 µg/plate<br>Vehicle: N/A<br>Duration of exposure: N/A<br>Guideline: similar to OECD 471[a]; GLP: N/A | No increase in mutation frequency was observed after SWCNT exposure. |
| ***b In vitro cytogenicity or micronucleus formation*** | | | | |
| Naya *et al.*, 2011<br>**KL1** | SWCNT<br>(AIST, Japan) | SSA: 1064 ± 37 $m^2/g$<br>Diameter: 3.0 ± 1.1 nm<br>Length: 1200 nm<br>Form of SWCNT: N/A | Type of assay: Chromosomal aberration test<br>Cell type: CHL/IU (fibroblast)<br>Metabolic activation: w and w/o S9<br>Concentrations: 0, 300, 500 and 1000 µg/mL<br>Vehicle: GIBCO<br>Duration of exposure: 6h (w and w/o S9) and 24h (w S9)<br>Guideline: OECD 473[a]; GLP: N/A | SWCNTs did not increase the number of structural (excluding gaps) or numerical chromosomal aberrations at any of the tested concentrations w or w/o metabolic activation. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Ema *et al.*, 2013 **KL1** | SWCNT (Nikkiso Co. Ltd. Tokyo, Japan; N-SWCNT) | SSA: 878 $m^2/g$<br>Diameter: 1.8 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: Chromosomal aberration test<br>Cell type: CHL/IU (fibroblast)<br>Metabolic activation: w and w/o S9<br>Concentrations: 6.25, 12.5, 25, 50 and 100 µg/mL<br>Vehicle: CMC-Na<br>Duration of exposure: 6h (w and w/o S9) and 24h (w/o S9)<br>Guideline: OECD 473[a]; GLP: N/A | The numerical chromosomal aberrations were not increased after short-term or continuous exposure with SWCNT. |
| Kim and Yu, 2014 **KL1** | SWCNT (Hanwha Nanotech, Incheon, Korea) | SSA: N/A<br>Diameter: 1-1.2 nm<br>Length: 20 µm<br>Form of SWCNT: N/A | Type of assay: Chromosome aberration test<br>Cell type: CHO-k1<br>Metabolic activation: w and w/o S9<br>Concentrations: 12.5-50 µg/mL<br>Vehicle: dispersion medium<br>Duration of exposure: 6h and 24h<br>Guideline: OECD 473[a]; GLP: N/A | SWCNTs did not produce a statistically significant increase in the number of cells with chromosome aberrations when compared with the negative control group at any of the dose levels tested, with or without metabolic activation. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Darne *et al.*, 2014 **KL2** | SWCNT[i] (Nanocyl; SWCNT 1100) | SSA: 1128 $m^2/g$ Diameter: 2 $(nm)^2$ Length: <1 (µm)2 Form of SWCNT: entangled | Type of assay: Fpg modified comet test Cell type: V79 (fibroblast) and SHE (epithelial) cells Metabolic activation: w and w/o Fpg enzyme Concentrations: 0.23 to 3.75 µg/$cm^2$ Vehicle: DMEM Duration of exposure: 24h Guideline: N/A; GLP: N/A | SWCNT induced no effect in either V79 or SHE cells, w or w/o Fpg treatment. No increase in ROS production was induced by exposure of either cell type to the SWCNT. |
| Kim and Yu, 2014 **KL2** | SWCNT (Hanwha Nanotech, Incheon, Korea) | SSA: N/A Diameter: 1-1.2 nm Length: ~20 µm Form of SWCNT: N/A | Type of assay: Comet test Cell type: Human PBL (lymphocyte) Metabolic activation: N/A Concentrations: 0, 25, 100 µg/mL Vehicle: RPMI-1640 Duration of exposure: 24, 48h Guideline: Singh *et al.,* 1988 protocol; GLP: N/A | After 24 and 48h, DNA damaging effects were observed to be higher after exposure to SWCNT in comparison with the control. DNA breakage was produced via the generation of reactive oxygen species (ROS). |
| Lindberg *et al.*, 2013 **KL2** | SWCNT[j] (SES Research, USA; product No.900-1351) | SSA: 436 $m^2/g$ Diameter: <2 nm Length: 1-5 µm Form of SWCNT: N/A | Type of assay: Cytokinesis-Block Micronucleus (CBMN) test Cell type: BEAS 2B (bronchial epithelial) Positive control: MMC Concentrations: 19-760 µg/mL Vehicle: BEGM Duration of exposure: 48 and 72 h Guideline: similar to OECD 487[a]; GLP: N/ | SWCNT was not able to produce chromosomal damage. |

[i] This study was also conducted with MWCNT.
[j] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Darne *et al.*, 2014 **KL2** | SWCNT[k] (Nanocyl; SWCNT 1100) | SSA: 1128 $m^2/g$ Diameter: 2 $(nm)^2$ Length: <1 (µm)2 Form of SWCNT: entangled | Type of assay: Cytokinesis-Block Micronucleus (CBMN) test Cell type: V79 (fibroblast) and SHE (epithelial) cells Positive control: MMS Concentrations: 0.23 to 3.75 $\mu g/cm^2$ Vehicle: DMEM Duration of exposure: 24h Guideline: OECD 487[a]; GLP: N/A | In V79 cells, SWCNT induced a significant increase in micronucleated cells at concentrations of 0.94 and 1.87 $\mu g/cm^2$ but not at 3.75 $\mu g/cm^2$. In SHE cells SWCNT had no effects. No increase in ROS production was induced by exposure of either cell type to SWCNT. |
| Lindberg *et al.*, 2013 **KL2** | SWCNT[l] (SES Research, USA; product No.900-1351) | SSA: 436 $m^2/g$ Diameter: <2 nm Length: 1-5 µm Form of SWCNT: N/A | Type of assay: Comet test Cell type: MeT-5A (mesothelial) and BEAS 2B (bronchial epithelial) Metabolic activation: N/A Concentrations: Exp1: 19-304 µg/mL Exp2: 304-760 µg/mL Vehicle: BEGM and RPMI Duration of exposure: 24 and 48 h Guideline: N/A; GLP: N/A | SWCNTs induced DNA damage in MeT-5A cells. Lower effect in BEAS 2B cells were observed. DNA adducts were induced by SWCNTs but these effects decreased after a 3-day exposure to SWCNTs, indicating that CNTs may lead to alterations in oxidative effects within the cells. |



[k] This study was also conducted with MWCNT.
[l] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Lindberg *et al.*, 2013<br>**KL2** | SWCNT[m]<br>(SES Research, USA; product No.900-1351) | SSA: 436 $m^2/g$<br>Diameter: <2 nm<br>Length: 1-5 µm<br>Form of SWCNT: N/A | Type of assay: Comet test<br>Cell type: MeT-5A (mesothelial) and BEAS 2B (bronchial epithelial)<br>Metabolic activation: N/A<br>Concentrations:<br>Exp1: 19-304 µg/mL<br>Exp2: 304-760 µg/mL<br>Vehicle: BEGM and RPMI<br>Duration of exposure: 24 and 48 h<br>Guideline: N/A; GLP: N/A | SWCNTs induced DNA damage in MeT-5A cells. Lower effect in BEAS 2B cells were observed. DNA adducts were induced by SWCNTs but these effects decreased after a 3-day exposure to SWCNTs, indicating that CNTs may lead to alterations in oxidative effects within the cells. |
| Toyokuni, 2013<br>**KL4**<br>(Source: Kisin *et al.*, 2007; KL2) | SWCNT<br>(CNI, Inc., Houston, TX) | SSA: 1040 $m^2/g$<br>Diameter: N/A<br>Length: 1–3 µm<br>Form of SWCNT: N/A | Type of assay: Comet test<br>Cell type: V79 (fibroblast)<br>Metabolic activation: N/A<br>Concentrations: 0, 24, 48 and 96 $\mu g/cm^2$<br>Vehicle: MEM<br>Duration of exposure: 3 and 24h<br>Guideline: N/A; GLP: N/A | A short-term SWCNT treatment for 3 h led to significant DNA damage (only at the highest SWCNT dose of 96 $\mu g/cm^2$. More prolonged treatment with SWCNT for 24 h increased all parameters of DNA damage in a concentration-dependent way. |
| Toyokuni, 2013<br>**KL4**<br>(Source: Jacobsen *et al.*, 2008;KL2) | SWCNT<br>(Thomas Swan, UK; EliCarb SWCNT) | SSA: 731 $m^2/g$<br>Diameter: 0.9–1.7 nm<br>Length: < 1 µm<br>Form of SWCNT: N/A | Type of assay: Fpg modified comet test<br>Cell type: FE1-MML (epithelial)<br>Metabolic activation: N/A<br>Concentrations: 100 µg/mL<br>Vehicle: DMEM F12<br>Duration of exposure: 3h<br>Guideline: N/A ; GLP: N/A | No induction of strand breaks and no change in cell mutant frequency but an increase in the level of FPG sensitive sites was observed after exposure to SWCNT |

[m] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013<br>**KL4**<br>(Source: Pacurari *et al.*, 2008; KL2) | SWCNT<br>(National Institute of Standards and Technology ,Gaithersburg, MD) | SSA: 293 $m^2/g$<br>Diameter: 0.8-2 nm<br>Length: < 1 µm<br>Form of SWCNT: N/A | Type of assay: Comet test<br>Cell type: Normal human mesothelial cell and malignant<br>Metabolic activation: N/A<br>Concentrations: 25 and 50 µg/$cm^2$<br>Vehicle: N/A<br>Duration of exposure: 24h<br>Guideline: N/A ; GLP: N/A | Exposure to SWCNT enhanced DNA damage in both cell lines at 25 and 50 µg/$cm^2$. |
| Toyokuni, 2013<br>**KL4**<br>(Source: Yang *et al.*, 2009; KL2) | SWCNT<br>(COCC, Chinese Academy of Science, Chengdu) | SSA: N/A<br>Diameter: 8 nm<br>Length: < 5 µm<br>Form of SWCNT: Rope-shaped CNT | Type of assay: Comet test<br>Cell type: Primary Balb/c mice embryo fibroblasts<br>Metabolic activation: N/A<br>Concentrations: 5 and 10 µg/mL<br>Vehicle: DMEM<br>Duration of exposure: 24h<br>Guideline: Singh *et al.,* 1988 protocol; GLP: N/A | Exposure to SWCNT increased DNA tail length and moment at 5 and 10 µg/mL. |
| Toyokuni, 2013<br>**KL4**<br>(Source: Lindberg *et al.*, 2009; KL2) | SWCNT<br>(Sigma Aldrich, Germany; no. 636797) | SSA: N/A<br>Diameter: 1.1 nm<br>Length: 0.5-100 µm<br>Form of SWCNT: N/A | Type of assay: Comet test<br>Cell type: BEAS2B (bronchial epithelial)<br>Metabolic activation: N/A<br>Concentrations: 0, 3.8, 19, 38, 76, 114, 228, 304 and 380 µg/mL<br>Vehicle: BEGM<br>Duration of exposure: 24, 48 and 72h<br>Guideline: N/A ; GLP: N/A | SWCNT induced a dose-dependent increase in DNA damage after 24h. After the 48-h and 72-h treatments with CNTs, the effect was also very clear; a statistically significant increase in DNA damage was observed at all tested doses. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013<br>**KL4**<br>(Source: Migliore *et al.*, 2010; KL4) | SWCNT[n]<br>(N/A) | SSA: 400 $m^2/g$<br>Diameter: 0.7-1.2 nm<br>Length: 0.5-100 µm<br>Form of SWCNT: N/A | Type of assay: Comet test<br>Cell type: RAW264.7 (macrophages)<br>Metabolic activation: N/A<br>Concentrations:<br>Exp1:0, 0.01, 0.1, 1, 10 and 100 µg/mL (2h, 24h)<br>Exp 2:0, 1, 10 and 100 µg/mL, 24h<br>Vehicle: N/A<br>Duration of exposure: 2 and 24h<br>Guideline: N/A; GLP: N/A | Effect of treatment with SWCNT were detectable at all concentrations tested (1–100 µg/mL). |
| Nikitina *et al.*, 2015<br>**KL4** | SWCNT<br>(Institute of Chemical Physics Problems, Russian Academy of Sciences, Chernogolovka). | SSA: N/A<br>Diameter: 1 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: Comet test<br>Cell type: HEF (fibroblast)<br>Metabolic activation: N/A<br>Concentrations: N/A<br>Vehicle: Eagle's medium<br>Duration of exposure: 3, 24 and 48 h<br>Guideline: N/A; GLP: N/A | The level of DNA damages increased after 3-h exposure to SWCNT. |
| Toyokuni, 2013<br>**KL4**<br>(Source : Sargent *et al.*, 2012; article not available) | SWCNT<br>(N/A) | SSA: 1040 $m^2/g$<br>Diameter: 1-4 nm<br>Length: 0.5-1 µm<br>Form of SWCNT: N/A | Type of assay: Chromosomal aberration test<br>Cell type: SAEC and BEAS-2B<br>Metabolic activation: N/A<br>Concentrations: 0, 0.024, 0.24, 2.4 and 24 µg/mL<br>Vehicle: N/A<br>Duration of exposure: 24h and 72h<br>Guideline: similar to OECD 473[a]; GLP: N/A | Increase in mitotic disruption was observed after SWCNT exposure. |

[n] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Nikitina *et al.*, 2015<br>**KL4** | SWCNT<br>(Institute of Chemical Physics Problems, Russian Academy of Sciences, Chernogolovka). | SSA: N/A<br>Diameter: 1 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: Chromosomal aberration test<br>Cell type: HEF (fibroblast)<br>Metabolic activation: N/A<br>Concentrations: N/A<br>Vehicle: N/A<br>Duration of exposure: 3, 24, or 48 h<br>Guideline: similar to OECD 473[a]; GLP: N/A | The level of DNA aberrations increased after 3-h exposure to SWCNT. |
| Toyokuni, 2013<br>**KL4**<br>(Source: Lindberg *et al.*, 2009; KL2) | SWCNT<br>(Sigma Aldrich, Germany; no. 636797) | SSA: N/A<br>Diameter: 1.1 nm<br>Length: 0.5-100 µm<br>Form of SWCNT: N/A | Type of assay: Cytokinesis-Block Micronucleus (CBMN) test<br>Cell type: BEAS2B (bronchial epithelial)<br>Positive control: Mitocyn C<br>Concentrations: 0, 3.6, 18, 36, 72, 144, 216, 288 and 360 µg/mL<br>Vehicle: BEGM<br>Duration of exposure: 24, 48h and 72h<br>Guideline: similar to OECD 487[a]; GLP: N/A | SWCNT did not increase the frequency of micronucleated cells at any dose after 24 or 72h. However, the 48-h treatment induced a significant increase in micronucleated cells at three different doses but a clear dose-dependency was not seen. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013<br>**KL4**<br>(Source: Cveticanin et al., 2009; KL3) | SWCNT[o]<br>(N/A) | SSA: N/A<br>Diameter: 1.1 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: Cytokinesis-Block Micronucleus (CBMN) test<br>Cell type: Human lymphocytes<br>Positive control: N/A<br>Concentrations: 25, 50, 100 and 150 µl/mL<br>Vehicle: PBmax-karyotyping<br>Duration of exposure: 68h<br>Guideline: similar to OECD 487[a]; GLP: N/A | SWCNT exposure enhance the incidence of micronuclei by 45.58% compared to the control and decrease the proliferation potential of cells. |
| Toyokuni, 2013<br>**KL4**<br>(Source: Migliore *et al.*, 2010; KL2) | SWCNT[p]<br>(N/A) | SSA: 400 $m^2/g$<br>Diameter: 0.7-1.2 nm<br>Length: 0.5-100 µm<br>Form of SWCNT: N/A | Type of assay: Cytokinesis-Block Micronucleus (CBMN) test<br>Cell type: RAW264.7 (macrophages)<br>Positive control: MMC<br>Concentrations: 0, 0.01, 0.10, 1, 10,100 µg/mL<br>Vehicle: Essential medium<br>Duration of exposure: 24h<br>Guideline: similar to OECD 487[a]; GLP: N/A | After SWCNT exposure, the number of micronucleated cells was significantly increased above the control at a concentration of 0.1 µg/mL and higher. |



[o] This study was also conducted with MWCNT.
[p] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013<br>**KL4**<br>(Source : Kisin *et al.*, 2007; KL2) | SWCNT<br>(CNI, Inc., Houston, TX) | SSA: 1040 $m^2/g$<br>Diameter: N/A<br>Length: 1–3 µm<br>Form of SWCNT: N/A | Type of assay: Cytokinesis-Block Micronucleus (CBMN) test<br>Species/strain/cell type: V79 (fibroblast)<br>Positive control: N/A<br>Concentrations: 12, 24, 48 and 96 $\mu g/cm^2$<br>Vehicle: MEM<br>Duration of exposure: 24h<br>Guideline: similar to OECD 487[a]; GLP: N/A | No significant increase in micronucleus induction was founded after SWCNT exposure. |
| Nikitina *et al.*, 2015<br>**KL4** | SWCNT<br>(Institute of Chemical Physics Problems, Russian Academy of Sciences, Chernogolovka) | SSA: N/A<br>Diameter: 1 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: Cytokinesis-Block Micronucleus (CBMN) test<br>Cell type: HEF (fibroblasts)<br>Positive control: N/A<br>Concentrations: N/A<br>Vehicle: Eagles's medium<br>Duration of exposure: 3, 24, or 48 h<br>Guideline: similar to OECD 487[a]; GLP: N/A | The incidence of micronuclei did not change after exposure to SWCNT. |
| ***c In vitro gene mutation in mammalian cells*** | | | | |
| Toyokuni, 2013<br>**KL4**<br>(Source: Pacurari *et al.*, 2008; KL2) | SWCNT<br>(National Institute of Standards and Technology ,Gaithersburg, MD) | SSA: 293 $m^2/g$<br>Diameter: 0.8-2 nm<br>Length: < 1 µm<br>Form of SWCNT: N/A | Type of assay: Histone H2AX phosphorylation of DNA double-strand breaks.<br>Cell type: normal and malignant mesothelial cells<br>Positive control: Crocidolite<br>Concentrations: 0, 25, 50 µg/cm2<br>Vehicle: BEGM<br>Duration of exposure: 24h<br>Guideline: N/A; GLP: N/A | Exposure to SWCNT increased H2AX phosphorylation. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013 **KL4** (Source:Cveticanin *et al.*, 2009; KL3) | SWCNT[q] (N/A) | SSA: N/A<br>Diameter: 1.1 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: Enumeration of γ H2AX foci in fibroblasts<br>Cell type: dermal fibroblast HDMEC (PromoCell)<br>Positive control: N/A<br>Concentrations: 0, 0.5, 30 µl/mL<br>Vehicle: DMEM<br>Duration of exposure: 24h<br>Guideline: N/A; GLP: N/A | SWCNTs induces Increased γ-H2AX foci (2.7-fold vs control). |
| ***d) In vivo mutagenicity*** | | | | |

[q] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Naya *et al.*, 2012 **KL1** | SWCNT (Nikkiso Co. Ltd., Japan; N-SWCNT) | SSA: 878 $m^2/g$<br>Diameter: 1.8 nm<br>Length: 4.4 µm<br>Form of SWCNT: entangled | Type of study: *In vivo* comet test on lung epithelial cells<br>Route of administration: intratracheal installation<br>Doses:<br>Exp1:0, 0.2, 1 mg/kg bw (single exposure)<br>Exp 2:0, 0.04, 2 mg/kg bw (repeated exposure)<br>Vehicle: 1% Tween 80 in PBS<br>Duration of treatment/exposure: single exposure (exp 1) and repeated exposure – once a week for 5 weeks (exp 2)<br>Positive control: EMS<br>Number of animals: 5 Crl: CD1 male rats/ group<br>Guideline: similar to OECD 489[a]; GLP: N/A | SWCNTs were not genotoxic in the comet assay following intratracheal instillation in rats. |
| Ema *et al.*, 2013 **KL1** | SWCNT (Nikkiso Co. Ltd. Tokyo, Japan; N-SWCNT) | SSA: 878 $m^2/g$<br>Diameter: 1.8 nm<br>Length: N/A<br>Form of SWCNT: N/A | Type of study: *In vivo* micronucleus test on erythrocyte<br>Route of administration: oral gavage<br>Doses: 5, 10, 20 mg/kg bw/day<br>Positive control: MMC<br>Vehicle: CMC-Na aqueous solution<br>Duration of treatment/exposure: 24h<br>Number of animals: 6 Crlj:CD1 male mice/ group<br>Guideline: OECD 474[a]; GLP: N/A | SWCNTs did not induce increases in MN frequency at any dose. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Kim and Yu, 2014<br>**KL1** | SWCNT<br>(Hanwha Nanotech, Incheon, Korea) | SSA: N/A<br>Diameter: 1-1.2 nm<br>Length: 20 µm<br>Form of SWCNT: N/A | Type of study*: In vivo* micronucleus test on erythrocyte<br>Route of administration: intraperitoneal<br>Doses: 0, 25 mg/kg, 50 mg/kg, 100 mg/kg<br>Positive control: MMC<br>Vehicle: dispersion medium<br>Duration of treatment/exposure: N/A<br>Number of animals: 6 ICR male mice / group<br>Guideline: 474; GLP: N/A | The SWCNTs did not evoke significant *in vivo* micronuclei frequencies in mice polychromatic erythrocytes at 25–100 mg/kg. |
| Naya *et al.*, 2011<br>**KL1** | SWCNT<br>(AIST, Japan) | SSA: 1064 ± 37 $m^2/g$<br>Diameter: 3.0 ± 1.1 nm<br>Length: 1200 nm<br>Form of SWCNT: N/A | Type of study: *In vivo* micronucleus test in bone marrow<br>Route of administration: oral gavage<br>Doses: 60 and 200 mg/kg/day<br>Vehicle: 1% Tween 80 in PBS<br>Positive control: MMC<br>Duration of treatment/exposure: once daily - 2 days<br>Number of animals: 5 Crlj:CD1 male mice / dose<br>Guideline: OECD 474[a]; GLP: N/A | SWCNTs did not show any potential for genotoxic activity in the *in vivo* micronucleus assay. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Honda *et al.*, 2017 **KL2** | SWCNT (Nikkiso Co., Ltd. Tokyo, Japan) | SSA: 878 $m^2/g$ **SWCNT long tubes:** Diameter: 2.4 ± 2.0 nm Length: 8.6 ± 4.3 **SWCNT short tube:** Diameter: 1.4 ± 0.7 nm Length: 0.55 ± 0.36 Form of SWCNT: entangled | Type of study: *In vivo* comet test on lung cells Route of administration: single intratracheal installation Doses: 0.2 or 1.0 mg/kg (long SWCNTs) or 1.0 mg/kg (short SWCNTs) Vehicle: PBS containing 1% salmon-DNA treatment/exposure: / Post exposure period: 26 weeks Number of animals: 5 F344/DuCrlCrlj male rats/ group Guideline: Similar to OECD 489[a]; GLP: N/A | No significant changes in the percent tail deoxyribonucleic acid were found in any group exposed to long and short SWCNT. |
| Toyokuni, 2013 **KL4** (Source: Jacobsen *et al.*, 2008; KL2) | SWCNT (Thomas Swan, UK; Elicarb) | SSA: 731 $m^2/g$ Diameter: 0.9-1.7 nm Length: < 1 µm Form of SWCNT: N/A | Type of study: *In vivo* comet test on BAL cells Route of administration: intratracheal Doses: 54 µg/animal Vehicle: N/A Duration of treatment/exposure: 3h Number of animals: $ApoE^{-/-}$ female mice Guideline: N/A; GLP: N/A | SWCNT exposure induced elevated level of DNA damage measured as % DNA in the tail. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013 **KL4** (Source: Folkmann *et al.*, 2009; KL3) | SWCNT (Thomas Swan; Elicarb) | SSA: 731 $m^2/g$ Diameter: 0.9-1.7 nm Length: < 1 µm Form of SWCNT: N/A | Type of study: Measurement of oxidatively modified DNA Route of administration: oral gavage Doses: 0.064 and 0.64 mg/kg Vehicle: N/A treatment/exposure: single exposure Number of animals: Fischer 344 female rats Guideline: N/A; GLP: N/A | Levels of 8-oxo-2'deoxyguanosine were increased in lung and liver at both doses. |
| Toyokuni, 2013 **KL4** (Source: Li *et al.*, 2007; KL3) | SWCNT (CNI, Houston) | SSA: 1040 $m^2/g$ Diameter: 1-4 nm Length: N/A Form of SWCNT: N/A | Type of study: Mitochondria DNA damage test Route of administration: pharyngeal aspiration Doses: 10 and 40 µg/mice Vehicle: N/A treatment/exposure: N/A Number of animals: C57BL/6 male mice Guideline: N/A; GLP: N/A | Increase in mitochondrial DNA damage (maximal at 7 days after exposure). |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Toyokuni, 2013<br>**KL4**<br>(Source: Shvedova *et al.*, 2008; KL3) | SWCNT<br>(CNI, Houston, TX) | SSA: 508 $m^2/g$<br>Diameter: 1-1.2 nm<br>Length: 0.1-1 µm<br>Form of SWCNT: N/A | Type of study: K-ras mutation analysis<br>Route of administration: inhalation and pharyngeal administration<br>Doses: 0, 5, 10, 20 µg/mouse (pharyngeal aspiration)<br>0, 5 $mg/m^3$ (inhalation)<br>Vehicle: N/A<br>treatment/exposure: 5h/day for 4 days (inhalation)//1, 7 and 28 days (pharyngeal aspiration)<br>Number of animals: C57BL/6 female mice<br>Guideline: N/A; GLP: N/A | Increased mutation frequency in mice was observed at 7 and 28 days post SWCNT exposure. |

### 4.6. Carcinogenicity

For the carcinogenicity, the database comprises 2 studies performed between 2011 and 2017, as summarized in **Table 6**. The studies used SWCNT with specific surface areas (SSA) ranging from 400 to 1000 $m^2/g$. They meet the criteria for Klimisch score 2 or 3.

The available reliable data indicate that SWCNTs did not induce a significant occurrence of malignant mesotheliomas, lung tumors.

**Table 6.** Available studies: carcinogenicity

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Honda *et al.*, 2017<br>**KL2** | SWCNT<br>(Nikkiso Co., Ltd. Tokyo, Japan) | SSA: 878 $m^2/g$<br>**SWCNT long tubes:**<br>Diameter: 2.4 ± 2.0 nm<br>Length: 8.6 ± 4.3<br>**SWCNT short tube:**<br>Diameter: 1.4 ± 0.7 nm<br>Length: 0.55 ± 0.36<br>Form of SWCNT: entangled | Type of study: *In vivo* carcinogenicity study<br>Route of administration: single intratracheal installation<br>Doses: 0.2 or 1.0 mg/kg (long SWCNTs) or 1.0 mg/kg (short SWCNTs)<br>Vehicle: PBS containing 1% DNA<br>Duration of treatment/exposure: /<br>Post exposure period: 26 ,52, 104-105 weeks<br>Number of animals: 60 F344/DuCrlCrlj male rats / group<br>Guideline: N/A; GLP: N/A | Most long SWCNT tubes deposited at the terminal bronchioles resulting in fibrosis and epithelium loss. the short SWCNTs appeared to reach the alveolus, resulting in chronic inflammatory responses. Neither the long nor the short SWCNTs induced a significant occurrence of malignant mesotheliomas, lung tumors. |

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Wang *et al.*, 2011b<br>**KL3** | SWCNT<br>(CNI, Houston, TX) | SSA: 400-1000 $m^2/g$<br>Diameter: 0.8-1.2 nm<br>Length: 0.1 µm<br>Form of SWCNT: N/A | Type of study: *In vivo* carcinogenicity screening<br>Route of administration: subcutaneous injection<br>Doses: 2 x $10^6$ SWCNT-transformed cells (B-SWCNT)<br>Vehicle: PBS<br>Duration of treatment/exposure: 7, 14 days<br>Number of animals: immunodeficient mice (nu/nu: Jackson Laboratory, Bar Harbor, ME)<br>Guideline: N/A; GLP: N/A | Immunodeficient mice were injected SWCNT transformed cells (B-SWCNT). B-SWCNT induce tumorigenesis in mice and exhibit an apoptosis resistant phenotype characteristic of cancer cells as compared to the control group. |

### 4.7. Neurotoxicity

For the assessment of neurotoxicity, the database comprises 5 studies performed between 2011 and 2019, as summarized in **Table 7**. The studies used SWCNT with specific surface areas (SSA) ranging from 380 to 700 $m^2/g$ and diameter ranging from 0.8 to 2 nm. They meet the criteria for Klimisch score 2 or 4.

The available identified data provide preliminary screening information suggesting that several types of SWNT possess the potential for neuro-related effects that could possibly be mediated by oxidative stress, apoptosis pathway as well as other pathways of cytotoxicity. The database is still very limited, especially when taking into account the many different particle types and their potential surface modifications. It follows that the insufficient database also hinders hazard identification and risk assessment on neurotoxicity of nanomaterials.

**Table 7.** Available studies: neurotoxicity

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| ***a) In vitro assays*** | | | | |
| Zeinabad *et al.*, 2016 **KL2** | SWCNT[r] (Neutrino Co., Tehran, Iran) | SSA: > 380 $m^2/g$<br>Outer diameter: 1-2 nm<br>Inner diameter 0.8-1.6 nm<br>Length: N/A<br>Form of SWCNT: entangled | Type of assay: *In vitro* neurotoxicity study<br>Cell type: PC12 (adrenal cells)<br>Concentrations:0, 0.01, 0.5, 2, 10, 50, 100 µg/mL<br>Vehicle: DMEM<br>Positive control: N/A<br>Duration of exposure: 48 hours<br>Guideline: N/A; GLP: N/A | SWCNT induced structural changes of tau protein (strong binding and static quenching). SWCNT also impaired the viability and complexity of PC12 cells in different modes of cytotoxicity. Apoptotic modes of cell death were activated in SWCNT-incubated cells. |
| Wang *et al.*, 2011a **KL2** | SWCNT (Beijing Nachen Technology & Development Co. Ltd. Beijing, China) | SSA: > 380 $m^2/g$<br>**Long SWCNT:**<br>Outer diameter: 1-2 nm<br>Length: 20 µm<br>**Short SWCNT:**<br>Outer diameter: 1-2 nm<br>Length: 0.5–2 µm<br>Form of SWCNT: N/A | Type of assay: *In vitro* neurotoxicity study<br>Cell type: PC12 (adrenal cells)<br>Concentrations: 5-600 µg/mL<br>Positive control: N/A<br>Vehicle: DMEM<br>Duration of exposure: 24 or 48h<br>Guideline: N/A; GLP: N/A | Cell viability, morphologic changes, DNA damage, apoptosis rate, redox homeostasis damage and MMP were affected by the presence of SWCNTs in a time and dose-dependent manner. Effects were suggested to be due to the decrease of endogenous antioxidants, the increase of ROS generation and the loss of transmembrane potential. |

[r] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| ***b) Ex vivo assays*** | | | | |
| Rasras *et al.*, 2019<br>**KL4** | SWCNT[s]<br>(Sigma Chemical Co., USA) | SSA: N/A<br>Outer diameter: 1-2 nm<br>Form of SWCNT: N/A | Type of assay: *Ex vivo* neurotoxicity study<br>Cell type: isolated rat brain mitochondria<br>Concentrations: 10, 20, and 40 µg/mL<br>Positive control: N/A<br>Vehicle: water<br>Duration of exposure: N/A<br>Guideline: N/A; GLP: N/A | Exposure to SWCNTs decreased mitochondrial survival and viability in a dose-dependent manner. SWCNTs could also damage mitochondrial membranes and induce the formation of ROS. Results suggest that SWCNTs likely damage brain tissue mitochondria by increasing oxidative stress and possibly activating the apoptosis pathway as well as other pathways of cytotoxicity. |
| ***c) In vivo assays*** | | | | |

[s] This study was also conducted with MWCNT.

| Reference/ KL rating | Test substance | Particle characterization | Methodology | Results |
|---|---|---|---|---|
| Gholamine *et al.*, 2017 **KL2** | SWCNT[t] (Synthesized at Research Institute of Petroleum Industry. Tehran, Iran) | SSA: 700 $m^2$/g<br>Outer diameter: 1-2 nm<br>Inner diameter 0.8-1.1 nm<br>Length: 10 µm<br>Form of SWCNT: N/A | Type of assay: *In vivo* neurobehavioral toxicity study<br>Species/strain/cell type: NMRI mice<br>Route of administration: single intraperitoneal injection<br>Concentrations: 80 or 800 mg/kg<br>Vehicle: PBS<br>Duration of exposure: 2 weeks<br>Number of animals: 10 mice/group<br>Guideline: N/A; GLP: N/A | Open field test did not yield significant group differences 24–29 days after SWCNT treatment. In elevated plus maze test, 800 mg/kg SWCNT showed an anxiogenic effect. The brain-derived neurotrophic factor gene expression in mice treated with 80 and 800 mg/kg SWNTs decreased as compared to the control group. Overall, SWCNT exposure may result in behavioral toxicity linked with expression of depression or anxiety. |
| Da Rocha *et al.*, 2019 **KL4** | SWCNT (Synthesized as per da Rocha *et al.*, 2013) | SSA: N/A<br>Diameter: N/A<br>Length: N/A<br>Form of SWCNT: N/A | Type of assay: *In vivo* neurotoxicity study<br>Species/strain/cell type: zebrafish<br>Route of administration: two intraperitoneal injection at 0 & 24 h<br>Concentrations: 30 mg/kg<br>Vehicle: SDS & Milli-Q water<br>Positive control: benzocaine<br>Duration of exposure: 48h<br>Number of animals: 60 animals/group<br>Guideline: N/A; GLP: N/A | SWCNT exposure increased between 3 and 6- fold the concentration of dopamine and serotonin. Similarly, a significant reduction in acetylcholinesterase activity was observed in the brains of SWCNT exposed zebrafish when compared to the control groups. |

[t] This study was also conducted with MWCNT.

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## 6. ANNEXES

### ANNEX 1 _ CHARACTERIZATION INFORMATION

| Single wall carbon nanotubes TUBALL™ | | |
|---|---|---|
| Name of nanoform | | Tuball™ |
| Number based particle size distribution | d10 | 1.2 - 1.45 nm |
| | d50 | 1.6 - 1.8 nm |
| | d90 | 1.9 - 2.2 nm |
| Shape and aspect ratio of particles | | Elongated tubes; length to diameter ratio 2000 – 10000:1 |
| Particle aggregation state | | Bundles of nanotubes |
| Particle agglomeration state | | Single wall carbon nanotubes are embedded in a matrix |
| Particle specific surface area | | See section 3.2 |
| Crystallinity | | Amorphous |
| Surface functionalisation / treatment | | No |
| Process | | Chemical vapor deposition (CVD) |
| Specific surface area | | 300 – 1500 $m^2/g$ |
| Additional information | | G/D range : ≥ 40 (RAMAN at 532 nm) |