For Official Use **ENV/CHEM/NANO(2015)30/ADD4**

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

28-Oct-2015

English - Or. English

ENVIRONMENT DIRECTORATE
CHEMICALS COMMITTEE

ENV/CHEM/NANO(2015)30/ADD4
For Official Use

Working Party on Manufactured Nanomaterials

Draft "Nano-SIAR": MWCNT

15th Meeting of the Working Party on Manufactured Nanomaterials

4-6 November 2015
OECD Conference Centre
Paris, France

Mar GONZALEZ
Tel. +(33-1) 45 24 76 96; Email: Mar.GONZALEZ@oecd.org

JT03385302

Complete document available on OLIS in its original format

English - Or. English

WPMN SPONSORSHIP PROGRAM INITIAL ASSESSMENT REPORT

FOR

MWCNT

1{a}. Chemical Name(s):	Multi-Walled Carbon Nanotubes
{1b. Category Name:}	
2. CAS Number(s):	308068-56-6 / 7782-42-5
3. Lead Sponsor:	Japan/ US
4. Shared Partnership with:	
5.Review and Quality Check Process Prior to CoCAM:	
6.Date of Submission:	June, 2015
7. Date of Last Update:	June, 2015
8. Comments:	

TABLE OF CONTENTS

IDENTITY

Identification of the Substance

CAS Number: 308068-56-6/ 7782-42-5
IUPAC Name:
Molecular Formula:
Structural Formula:
Molecular Weight:
Synonyms:

Purity/Impurities/Additives

Some MWCNTs contain catalytic metals as impurities. Nikkiso MWCNT's purity is more than 98 % and the others' were more than 92 to 95%.

Table 1 Summary of purity and impurities of tested MWCNTs

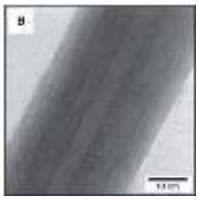
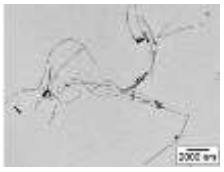
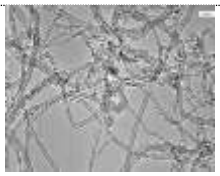
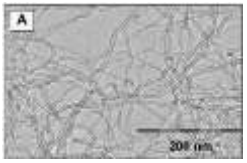
	purity	impurities
Nikkiso MWCNT	> 98%	Ca, Al, Fe
Arkema Graphistrength C100	> 92%	
Nanocyl NC7000	> 90%	
Mitsui MWNT-7	> 95%	Fe, Cr, Ni
Baytubes	> 95%	
Hanwha MWCNT	95%	Fe, Co, Al2O3

Physical-Chemical Properties

Physical Chemical properties of tested MWCNTs are summarized in Table 2 and Table 3.

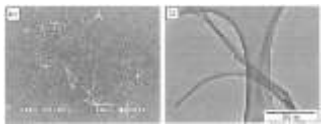
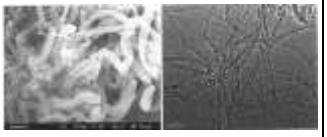
Table 2 Summary of Physical-Chemical Properties of principal MWCNTs

		Nikkiso MWCNT	Graphistrength C100	Nanocyl NC7000
1. Agglomeration / aggregation	method	SEM	SEM	
				
2. Water solubility/ Dispersability		n/a	n/a	n/a
3. Crystalline	method	TEM		

		Nikkiso MWCNT	Graphistrength C100	Nanocyl NC7000
phase	Results			
	method	Raman Spectoscopy.		
	Results	G/D ratio = 8 +/- 3		
4. Dustiness	method	Vortex Shaker method		DIN 55992-2
	Results	Respirable mass conc. = 0.061 mg/m3		No nanoscale particle above ambient levels. No respirable particles.
5. Crystallite size	method	TEM	TEM	TEM
	Results	Dia = 48nm, (SD=1.1nm) L = 0.94 µm, (SD=2.3µm)	Average internal diameter: 4.8 nm, Average external diameter: 11.7 nm Average length: 1097 nm, Avergae number of walls: 10	External diameter distribution from 5 to 15 nm, with a mean value of 9 nm Length distribution from 0.1 to 10µm with a mean value of 1.5 µm
6. Representative TEM picture(s)	method	TEM		TEM
	Results			
7. Particle size distribution	method	TEM, SEM	Internal Method (NTC.DTC 06)	Malvern on dry powder
	Results	Number ratio of Principal MWCNT used for Inhalation test -Individual existence: 72% -Bundle-like: 18% -Agglomeration: 10%	D(v, 0.5) = 416.2µm	D (v, 0.1) 31.6µm D(v, 0.5)= 85 µm D(v,0.9)= 228 µm
8. Specific surface area	method	BET: ISO 9277: 1955	CA.MDA.050/ Internal method	BET: DIN/ISO9277
	Results	69.4 m2/g	212 m2/g	230 m2/g
9. Zeta potential (surface charge)	method	electrophoretic mobility		
	Results	-14.7 +/- 0.9 mV		
10. Surface chemistry	method	XPS.		
	Results	O/C = 0.004		
11. Photocatalytic activity	method	ISO 22197-1 or JIS R 1701 "Test method of air purification performance of semiconducting photocatalytic materials"		
	Results	no photocatalytic activity		
12. Pour density	method	ASTMD 1513-05	CaMDA 053 Mercury porosity	DIN/ISO 9136

		Nikkiso MWCNT	Graphistrength C100	Nanocyl NC7000
	Results	0.0038 g/cm^3	0.09 g/cm^3	0.06 g/cm3
13. Porosity	method	JIS K 1150-1994/ISO 15901-1		
	Results	Pore volume: 24.6 ml/g, Davg = 420 nm		
14. n-Octanol-water partition coefficient		n/a	n/a	n/a
15. Redox potential				
16. Radical formation potential				

Table 3 Summary of Physical-Chemical Properties of alternate MWCNTs

		Mitsui MWNT-7	Baytubes	Hanwha CM100
1. Agglomeration/ aggregation	method	Cascade inpacter	REM	
	Results	MMAD: 1.5µm, GSD 1.67µm		
2. Water solubility/ Dispersability	method			Oxidized MWCNT solution was diluted with deionised water and filtered.
	Results			Oxidised MWCNT solution was completely dispersed.
	method			MWCNT was refluxed with 3:1-mixture of concentrated nitric acid and sulfuric acid. After refluxing, the MWCNT solution was neutralized and dispersed with deionised water.
	Results			Macroscopic dispersibility of MWCNTs was less than 7% when dispersed with deionised water. When MWCNTs dispersed with 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) solution, macroscopic dispersibility of MWCNTs was increased to 18%
3. Crystalline phase	method	High resolution TEM		Raman spectroscopy
	Results			ID/IG of grown MWCNTs was 1.238; this result represented MWCNTs contained high levels of defects
4. Dustiness	method		EN-15051-B	
	Results		respirable dustiness was low	
5. Crystallite size	method	SEM	TEM	TEM and DLS
	Results	Dia = 88nm, (SD=5nm) L = 5.0 µm, (SD=4.5µm)	d50=11nm tube length 380-902 nm	Diameter: 10 to 15 nm Length: less than 20 µm
6. Representative TEM picture(s)	method	TEM, SEM		FESEM
	Results			
7. Particle size	method	TEM	SEM	DLS

		Mitsui MWNT-7	Baytubes	Hanwha CM100
distribution	Results	Diameter: 70-170 nm, Length: 1-19μm (>5μm : 27.5%)	400 μm	The main range of length distribution was 543.3 ± 230 and 10451 ± 8421.6 nm, respectively
8. Specific surface area	method	BET	BET	BET
	Results	23 m2/g	253 m2/g	224.9 m^2/g
9. Zeta potential (surface charge)	method		Zeta-potentials were determined at different pH values (2-11) with a Malvern Zetasizer	ELS (electrophoretic light scattering) spectrometer, FT-IR
	Results		The iso-electric point was between pH 5-6.	-10mV ~ -45mV.
10. Surface chemistry				
11. Photocatalytic activity				
12. Pour density	method		DIN/ISO 9136	
	Results		0.16 g/cm2	
13. Porosity	method		The agglomerate density were measured using the Hg-porosimety method. Instrument: PASCAL 140/440 from Thermo Electron Corp.	
	Results		Daggl = 0.32 g/cm³	
14. n-Octanol-water partition coefficient		n/a	n/a	n/a
15. Redox potential				
16. Radical formation potential	Test method	Electron spin resonance (ESR) measurements		
	Results	when MWCNT were substituted for Fe2+ in the reaction, no •OH generation was detected, indicating the iron present in MWCNT was not capable of generating measurable ROS.		

GENERAL INFORMATION ON EXPOSURE

Environmental Exposure and Fate

Photodegradation

No information is available.

Stability in Water

No information is available.

Transport between Environmental Compartments

No information is available.

Biodegradation

Two readily biodegradability tests were conducted with **Nikkiso MWCNT** according to OECD test guideline in compliance with GLP.

A biodegradation test according to OECD test guideline 301F (Maonometric respirometry method) was conducted (AIST 2011, CERI 15605)[1]. The concentration of **Nikkiso MWCNT** was 100 mg/L and the concentration of the activated sludge was 30 mg/L as suspended solid matter. Biodegradation by BOD after 28 day cultivation period was 0 %. A biodegradation test according to OECD test guideline 301C (Modified MITI method) was conducted with cultivation period of 28 days (AIST 2011, CERI 15621 [Nikkiso MWCNT Biodegradation 301C])[2]. Biodegradation by BOD after 28 day cultivation period was 0 %.

A biodegradation test according to OECD test guideline 301F (Maonometric respirometry method) was conducted with **Arkema Graphistrength C100** (Arkema, Dossier 5.2.1 Graphistrength C100 Biodegradation TG 301F)[3]. The result showed no mineralization.

Concerning the inherent bio-degradability, one GLP test was conducted with Nikkiso MWCNT. A biodegradation test according to OECD test guideline 302C (Modified MITI method II) showed 1 % biodegradation after 28 day cultivation period (AIST 2011, CERI 15622 [Nikkiso MWCNT Biodegradation TG302C])[4]. The concentration of Nikkiso MWCNT was 30 mg/L and the concentration of the activated sludge was 100 mg/L as suspended solid matter.

Aerobic biodegradation test methods are designed for measurement of oxidation of organic substances. As MWCNTs are not an organic substance, biodegradation does not occur based on these test methods. Based on the results above, it is thought that MWCNTs were not readily bio-degradable.

As additional information, **Baytubes** was reported not readily biodegradable by OECD TG 301 test[5].

Bioaccumulation

No information is available.

HAZARDS TO THE ENVIRONMENT

Aquatic Effects

Acute and chronic toxicity studies of MWCNTs to aquatic species from three trophic levels are available.

Acute Toxicity Test Results

Fish

Data on the acute toxicity to fish are available for MWCNTs. Reliable studies are summarized in Table 4-1.

An acute toxicity test of **Nikkiso MWCNT** was conducted with Japanese Medaka, *Oryzias latipes* according to OECD test guideline 203 in compliance with GLP (AIST 2011, CERI 95399 [Nikkiso MWCNT (TG 203) Short-term toxicity to fish.001])[6]. Seven fish were exposed in static system at nominal concentrations of 10 mg/L. As a vehicle for the preparation of the test water, 5-time amount of polyoxyethylene castor oil (HCO-40) was added to Nikkiso MWCNT. The mechanical stirring was conducted in order to disperse the substance in the test water. Test concentration of 10 mg/L was decided taking the upper limit of the vehicle concentration into consideration. No mortality was observed at the concentrations of 10 mg/L after 96 hours. A 96-hour LC_{50} of >10 mg/L was determined.

An acute toxicity test of **Arkema Graphistrength C100** was conducted according to OECD test guideline 203 (Akema, [Graphistrength C100 (TG 203) Short-term toxicity to fish.001])[7]. No dispersing agents to stabilize the dispersion of the substance in the water column, but used mechanical stirring to keep the substance in suspension. A 96-hour LC_{50} was determined to be >100 mg/L which was the highest concentration.

As a prolonged toxicity test, a fourteen days toxicity test with **Nikkiso MWCNT** was carried out with Japanese Medaka, Oryzias latipes according to OECD test guideline 204 under flow-through conditions in compliance with GLP (MOE, 2011d [Nikkiso MWCNT (TG 204) Long-term toxicity to fish.001])[8]. Ten fish were exposed in semi-static system at nominal concentrations of 0.10, 0.32, 1.0, 3.2 and 10 mg/L. Concentrations of Nikkiso MWCNT in the test water were not measured during the exposure period. As a vehicle, Tween 80 was used with the concentration of 100 mg/L in the test water. Based on the behavior and mortality, a 14-day LC50, 14-day NOEC and 14-day LOEC were >10 mg/L, 3.2 mg/L and 10 mg/L, respectively.

Table 4-1 Summary of Acute Toxicity to Fish (nominal concentration)

Test substance	Species	Method	Result	Reference
Nikkiso MWCNT	*Oryzias latipes*	OECD TG 203	96-hour LC_{50} >10 mg/L	AIST 2011, CERI 95399
Arkema Graphistrength C100	*Danio rerio*	OECD TG 203	96-hour LC_{50} >100 mg/L	Arkema 2010, REACH Dossier
Nikkiso MWCNT	*Oryzias latipes*	OECD TG 204 Semi-static system	14-d NOEC: 3.2 mg/L (weight) 14-d LOEC: 10 mg/L(weight) 14-d EC_{50}: >10 mg/L	AIST 2011, MCM A100701

As additional information, 14-d LOEC of **Nanocyl NC700**0 by OECD TG 204 was reported > 100 mg/L(weight)[9] and 96-hour LC_5 **of Baytubes** by OECD TG 203 was reported >100 mg/L[5].

Invertebrate

Data on the acute toxicity to aquatic invertebrates are available for MWCNTs. Reliable studies are summarized in Table 4- 2.

Daphnia magna were exposed to **Nikkiso MWCNT** at nominal concentrations of 10 mg/L for 48 hours in a static system according to OECD test guideline 202 in compliance with GLP (AIST 2011, CERI

95398)[10]. Immobilization was 0 % after 48 hours both for concentration of 10 mg/L and for the vehicle control. Not only immobilization but also any other symptom was observed after 48 hours both for concentration of 10 mg/L and for the vehicle control. As a vehicle for the preparation of the test water, 5-time amount of polyoxyethylene castor oil (HCO-40) was added to Nikkiso MWCNT. The mechanical stirring was used in order to disperse the substance in the test water. Test concentration of 10 mg/L is decided taking the upper limit of the vehicle concentration into consideration. A 48-hour EC_{50} of >10 mg/L was determined.

An acute immobilization test of **Arkema Graphistrength C100** was conducted according to OECD test guideline 202 (Arkema, [Graphistrength C100 (TG 202) Short-term toxicity to aquatic invertebrates.001])[11]. No dispersing agents to stabilize dispersion of MWNCT in the water column, but used mechanical stirring to keep MWCNT in suspension. 48-hour EC_{50} was determined to be >100 mg/L.

Table 4- 2 Summary of Acute Toxicity to Aquatic Invertebrates

Test substance	Species	Method	Result	Reference
Nikkiso MWCNT	*Daphnia magna*	OECD TG 202 Static	48-h EC_{50} >10 mg/L, immobilization	AIST 2011, CERI 95398
Arkema Graphistrength C100	*Daphnia magna*	OECD TG 202	48-h EC_{50} >100 mg/L, immobilization	Arkema 2010, REACH dossier

As additional information, 48-h EC_{50} of both **Nanocyl NC700**0 and **Baytubes** by OECD TG 202 were reported > 100 mg/L [5, 9].

Aquatic plant, e.g. Algae

Data on the acute toxicity to aquatic plants are available for MWCNTs. Reliable studies are summarized in Table 4-3.

An alga growth inhibition test with **Nikkiso MWCNT** was conducted according to OECD test guideline 201 in compliance with GLP (AIST 2011, CERI 95397)[12]. *Pseudokirchneriella subcapitata* were exposed to Nikkiso MWCNT for 72 hours at nominal concentrations of 0.10, 0.32, 1.0, 3.2 and 10 mg/L. As a vehicle, 5-time amount of polyoxyethylene castor oil (HCO-40) was added to Nikkiso MWCNT. The mechanical stirring was used in order to disperse the substance in the test water. The highest concentration of 10 mg/L was decided taking the upper limit of the vehicle concentration into consideration. A 72-hour EC_{50} obtained on the basis of growth rate was > 10 mg/L.

An alga growth inhibition test with **Arkema Graphistrength C100** was conducted according to OECD test guideline 201 in compliance with GLP (Arkema, [Graphistrength C100 (TG 201)Toxicity to aquatic algae and cyanobacteria.002])[13]. *Pseudokirchneriella subcapitata* were exposed to Arkema Graphistrength C100 for 72 hours with nominal concentrations between 10 and 1000 mg/L. 72-hour EC_{50} was 19 mg/L.

Table 4- 3 Summary of Acute Toxicity to Aquatic Plants

Test substance	Species	Method	Result	Reference
Nikkiso MWCNT	*Pseudokirchneriella subcapitata*	OECD TG 201 Static	72-h EC_{50} : >10 mg/L, growth rate	AIST 2011, CERI 95397
Arkema Graphistrength	*Pseudokirchneriella subcapitata*	OECD TG 201	72-h EC_{50}: 120 mg/L, growth rate	Arkema 2010, REACH dossier

C100				

As additional information, 72-h EC_{50} of **Nanocyl NC700**0 and **Baytubes** by OECD TG 201 were reported 8.4 mg/L [9].and 134 mg/L [5] respectively.

Chronic Toxicity Test Results

Invertebrates

Data on the chronic toxicity to aquatic invertebrates are available for MWCNTs. Reliable studies are summarized in Table 4-4.

A chronic toxicity test with *Daphnia magna* was conducted with **Nikkiso MWCNT** according to OECD test guideline 211 in compliance with GLP (AIST, 2011 [Nikkiso MWCNT (TG211) Long-term toxicity to aquatic invertebrates.001])[14]. Exposure period was 21 days under semi-static conditions (test water was renewal every 2 days). The trial test was performed at a concentration range from 0.10 to 10 mg/L, but the mortality and abnormality of parents *Daphnia magna* were seen even at the lowest concentration of 0.10 mg/L. Therefore themain test was performed at the maximum concentration of 0.10 mg/L. Ten daphnia were exposed at nominal concentrations of 0.0030, 0.0095, 0.030, 0.095 and 0.30 mg/L. As a vehicle, Tween 80 was used with the concentration of 3.0 mg/L in the test water. Concentrations of Nikkiso MWCNT in the test water were not measured during the exposure period. Values of 21-day EC_{50}, NOEC, LOEC were > 0.30 mg/L, 0.3 mg/L, > 0.30mg/L respectively.

A chronic toxicity test with *Daphnia* magna was conducted with **Arkema Graphistrength C100** according to OECD test guideline 211 in compliance with GLP (Arkema, [Graphistrength C100 (TG211) Long-term toxicity to aquatic invertebrates.001])[15]. Exposure period was 21 days under semi-static conditions. Suspensions were renewed every two or three days, and stirring of the suspension was ensured. Test concentrations were ranged from 5 to 100 mg/L. Values of 21-day EC_{50} and NOEC, LOEC were 317.75 mg/L, 47 mg/L, 100 mg/L, respectively.

Table 4- 4 Summary of Chronic Toxicity to Aquatic Invertebrates (nominal concentration)

Test substance	Species	Method	Result	Reference
Nikkiso MWCNT	*Daphnia magna*	OECD TG 211 semi-static	21-d NOEC: 0.3 mg/L 21-d LOEC: > 0.3 mg/L 21-d EC_{50}: > 0.3 mg/L	AIST 2011, MCM A 100701
Arkema Graphistrength C100	*Daphnia magna*	OECD TG 211 semi-static	21-d NOEC: 47 mg/L 21-d LOEC: 100 mg/L 21-d EC_{50}: 317.75 mg/L	Arkema 2010, REACH dossier

As additional information, 14-d LOEC of **Nanocyl NC700**0 by OECD TG 211 was reported > 25 mg/L [9].

Aquatic plant, e.g. Algae

Data on the chronic toxicity to aquatic plants are available for MWCNTs. Reliable studies are summarized in Table 4-5.

Corresponding to the algal growth inhibition test (AIST, 2011 [Nikkiso MWCNT T(TG201) oxicity to aquatic algae and cyanobacteria.001])[16], which is described in the section of acute toxicity, a chronic ecotoxicity of **Nikkso MWCNT** was also determined. A 72-hour NOEC of Nikkiso MWCNT obtained on the basis of growth rate was 0.32 mg/L.

An alga growth inhibition test under GLP compliance with **Arkema Graphistrength C100** which is explained in the acute toxicity area determined a 72-hour ErC10 7.8 mg/L (Arkema, [Graphistrength C100 (TG201) Toxicity to aquatic algae and cyanobacteria.002])[17].

Table 4- 5 Summary of Chronic Toxicity to Aquatic Plants (nominal concentration)

Test substance (CAS No.)	Species	Method	Result (mg/L)	Reference
Nikkiso MWCNT	*Pseudokirchneriella subcapitata*	OECD TG 201 Static	72h NOEC: 0.32, growth rate	AIST 2011, CERI 95397
Arkema Graphistrength C100	*Pseudokirchneriella subcapitata*	OECD TG 201	72h ErC10: 7.8 mg/L, growth rate	Arkema 2010, REACH dossier

Toxicity to Microorganisms

Data on the toxicity to microorganisms are available for MWCNTs. Reliable studies are summarized in Table 4-6.

An activated sludge, respiration inhibition test was conducted with **Nikkiso MWCNT** according to OECD test guideline 209 in compliance with GLP (AIST, 2011 [Nikkiso MWCNT (TG 209) Effect on activated sludge at WWTP.001])[18]. Activated sludge using this test was obtained from a local waste-water treatment plant. Test concentration of Nikkiso MWCNT was 100 mg/L as suspended solid matter. Oxygen uptake rates were measured in order to decide the inhibition of respiration of the microorganisms. A 3-hour EC_{50} of Nikkiso MWCNT based on the respiration inhibition was determined to be > 100 mg/L

An activated sludge, respiration inhibition test was conducted with **Arkema Graphistrength C100** according to OECD test guideline 209 (Arkema, [Graphistrength C100 (TG 209) Effect on activated sludge at WWTP])[19]. Test concentrations were 500 mg/L and 5,000 mg/L. A 3-hour EC_{50} of Graphistrength C100 based on the respiration inhibition was determined to be > 5,000 mg/L.

Table 4- 6 Summary of Toxicity to Microorganisms

Test substance	Species	Method	Results	Reference
Nikkiso MWCNT	activated sludge	OECD TG 209	3-hour EC_{50}: > 100 mg/L, respiration inhibition	AIST 2011, CERI 95936
Arkema Graphistrength C100	activated sludge	OECD TG 209	3-hours EC_{50}: > 5,000mg/L, respiration inhibition	Arkema 2010, REACH dossier

As additional information, 3-h EC_{50} of **Baytubes** by OECD TG 209 was reported > 10000 mg/L [9].

Sediment-water toxicity

No data are available on sediment-water toxicity.

Terrestrial Effects

One reliable study on the terrestrial effects is available for MWCNTs shown in Table 4-7.

A microorganism toxicity test with **Nkkiso MWCNT** was conducted according to OECD test guideline 216 "Sol Micro-organisms: Nitrogen Transformation Test" in compliance with GLP. Concentration of Nikkiso MWCNT in the soil was 1,000 mg/dry-kg, and exposure duration was 28 days. Soil used in the test was clay loam, sand content was 53.5 %, pH was 5.4, organic carbon content was 0.9 % and biomass carbon was 88 mg/kg. A 28-day EC_{50} based on the inhibition of nitric acid synthesis was determined to be > 100 mg/kg soil dw (AIST, 2011 [Nikkiso MWCNT (TG 216) Toxicity to soil microorganisms.001])[20].

Table 4- 7 Summary of Terrestrial Effects

Test substance	Species	Method	Results	Reference
Nikkiso MWCNT	Micor-organisms in soil	OECD TG 216	28-day EC_{50}: > 100 mg/L, inhibition of nitric acid synthesis	AIST 2011, MCM A100703

Other Environmental Effects

A micro-nucleus assay (*Xenopus laevi*) was conducted with **Arkema Graphistrength C100** according to ISO 21427-1 (Arkema, [Graphistrength C100 Xenopus laevi micronucleus assay (ISO21427-1)])[21]. The result showed no genotoxic effects.

Conclusion

Acute toxicity

Acute aquatic toxicity data are available for some of MWCNTs. For fish, 96-hour LC_{50} values are > 10 mg/L for **Nikkiso MWCNT** and > 100 mg/L for **Arkema Grphistrengh C100**. For daphnids, 48-hour

EC_{50} values are > 10 mg/L for **Nikkiso MWCNT** and > 100 mg/L for **Arkema Grphistrengh C100**. For algae, 72-hour EC_{50} values are > 10 mg/L for **Nikkiso MWCNT** and =19 mg/L for **Arkema Grphistrengh C100**.

The tests with **Nikkiso MWCNT** were conduced with a vehicle and the mechanical stirring was used in order to disperse the substance in the test water. Concerning the test with Arkema Grphistrengh C100, no dispersing agents were used to stabilize the dispersion of the substance in the water, but mechanical stirring was used to keep the substance in the test water.

Chronic toxicity

Chronic aquatic toxicity data are available for some of MWCNTs. For fish, a value of 14-day NOEC with **Nikkiso MWCNT** according to OECD test guideline 204 is 3.2 mg/L. A vehicle was used for this test.

For daphnids immobilization test, values of 21-day NOEC are 0.3 mg/L for **Nikkiso MWCNT** and 47 mg/L for **Arkema Grphistrengh C100**. A vehicle was used for the test with Nikkiso MWCNT. For the test with Arkema Grphistrengh C100, suspensions were renewed every two or three days, and stirring of the suspension was ensured.

For algae, 72-hour NOEC values are 0.32 mg/L for **Nikkiso MWCNT** and 10 mg/L for **Arkema Grphistrengh C100**.

Toxicity to micro-organisms

Based on OECD test guideline 209, values of 3-hour EC_{50} were > 100 mg/L and 5000 mg/L for **Nikkiso MWCNT** and **Arkema Graphistrengh C100** respectively.

Sediment-water toxicity

No data are available on sediment-water toxicity.

HUMAN HEALTH HAZARDS

Effects on Human Health

Toxicokinetics, Metabolism and Distribution

Studies in Animals

In vitro Studies

No information is available.

In vivo Studies

There are several reliable information on distribution and elimination of MWCNT when administered by inhalation and intratracheally in rats. As no reliable information, orally or intravenously administrated studies are also available.

Male Wistar rats were exposed to **Nikkiso MWCNT** aerosol through whole body inhalation on 6 hrs/day, 5 days/week for 4 weeks. The average concentration of MWCNT was 0.37 mg/m3, and Triton X-100 as a vehicle control and unexposed group were set simultaneously. After the completion of inhalation 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). As a result, 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 life times of MWCNT were 51 days (XRD) and 54 days (ECA), respectively [Oyabu, T. et al., 2011; Nikkiso MWCNT : Basic toxicokinetics: 001][22].

As additional information, an inhalation study for **Mitsui MWNT-7** was conducted with a newly designed direct injection system that generated well-dispersed aerosol. Mice were exposed to MWCNT by inhalation for 2 hour a day for 5 days. In the peripheral alveolar space, single fibers were found phagocytized in alveolar macrophages [Taquahashi et al., 2013][23]. In another study, Mitsui MWNT-7 exposed by aerosol inhalation (at 5 mg/m3 for 6 h) was detected in the lung of rats up to 56 days after the exposure. As for intratracheal administration of 2 μg Mitsui MWNT-7, 0.68 μg, 0.96 μg and 0.34 μg were collected from the right lung, left lung and trachea respectively [Ohnishi, 2013][24].

Male F344 rats intratracheally instilled with **Mitsui MWNT-7** suspended in phosphate-buffered saline containing 0.1% Tween 80 at doses of 0(vehicle), 40 or 160 μg/rat. They were sacrificed on days 1, 7, 28 or 91 after instillation, and light microscopic examinations were performed on lung-associated lymph nodes (LALN) tissues. As a result, MWCNT instilled intratracheally was translocated to right and left posterior mediastinal lymph nodes. Deposition of MWCNT was greater in the posterior mediastinal lymph node than in the parathymic lymph node, and amount of MWCNT deposited in these two lymph nodes increased gradually and dose-dependently with time during the 91-day post exposure period. MWCNT was phagocytosed by nodal macrophages, and some of the MWCNT-laden macrophages were aggregated [Aiso, S. et al., 2011; Mitsui MWNT-7: Basic toxicokinetics: 001] [25].

Information on oral absorption of MWCNT is available from the acute dose toxicity studies in rats. In one study[33] given oral doses up to 2,000 mg/kg bw, no deaths occurred in spite of some toxic clinical signs

observed. In the other study[32] given up to 200 mg/kg bw, no effects were seen except for black feces. From these results, it was considered that oral absorption of MWCNT is not so well.

The following study was conducted with functionalized MWCNT-COOH and is treated as reference information.

In the frame of the Nanogenotox program [Jacobsen et al., 2013][26], eighteen male (n=18) and 12 female (n=12) rats received a single (day 1) or repeated (on 5 consecutive days, day 1-5) oral or intravenous (IV) administrations of ^{14}C labelled **Graphistrength C100** (named NM 402 in the report) and **Nanocyl NC7000** (NM 400 in the report) dispersed in 0.05 wt% Rat Serum Albumin (RSA) in ultra pure water.^{14}C-Carboxylation of carbon nanotubes was carried out using a three steps chemical process [Georgin et al., 2009] [27]. Six (n=6) male and three (n=3) female animals were treated with the vehicle used for the dispersion. The single dose groups received a dose per animal between 9.6 – 10 mg/kg b.w. for male animals, and 10.9 – 11.3 mg/kg b.w. for female animals depending on the actual weight of the animal. The repeated dose groups received a total cumulative dose per animal between 48 – 50 mg/kg b.w after 5 days treatment for male animals, and 54.5 – 56.5 mg/kg b.w. for female animals depending on the actual weight of the animal.

After oral administration, ^{14}C labelled MWCNTs didn't show translocation from the GI-tract into the systemic circulation or any of the organs investigated (including spleen, liver, and lung).
In blood obtained at 24 hours after the IV administration of the^{14}C-Graphistrength C100 very low to almost no radioactivity was detected whatever the protocol of administration in either male or female rats. However, for Nanocyl NC7000 at 24 hours after the IV administration both for the single and repeated dose a considerable level of ^{14}C was measured up to 10% of the injected dose.
After a single IV dose of ^{14}C-Graphistrength C100 to male rats only 8% of the injected dose was observed in organs at day 1, with the liver being the main target organ (7%). In females the recovery was 24% after a single dose. After a single IV dose of ^{14}C-Nanocyl NC7000 to male rats most of the injected dose was observed in liver (24%) and lung (25%) at day 1, with a few percent present in spleen, kidneys, heart and testes. The ^{14}C-Nanocyl NC7000 showed much higher bioaccumulation than ^{14}C-Graphistrength C100.

In general, charged particles (such as carboxylated polystyrene nanoparticles [Jani et al. 1989][28] or those composed of positively charged polymers) exhibit poor oral bioavailability through electrostatic repulsion and mucus entrapment.

Studies in Humans

In vitro Studies

No information on humans is available.

In vivo Studies

No information on humans is available.

Conclusion

When exposed by whole body inhalation for 4 weeks (6 hrs/day, 5 days/week) at 0.37 mg/m3 in rats, approximately 20% of MWCNT inhaled were deposited in the lung, and eliminated with half life of 51-54 days post exposure. MWCNT intratracheally instilled was translocated from the lung to lung associated lymph nodes, but there was no evidence to distribute systemic when inhaled. Oral absorption rate of MWCNT was considered as not significant.

Acute Toxicity

Studies in Animals

Inhalation

Two reliable study reports are available. The details of the studies are as follows.

The first study was performed according to OECD test guideline (No. 403). Wistar rats were nose-only exposed for 6 hrs by inhalation to **Baytubes** at concentrations of 11 and 241 mg/m^3. Inflammatory endpoints in bronchio-alveolar lavage (BAL) were determined on post-exposure days 7, 28 and 90. The deposition of cobalt (tracer as an impurity of Baytubes) was determined in lungs, lung-associated lymph nodes (LALN), brain, kidneys, testes and liver. No deaths occurred. The changes in BAL of exposed rats regressed over time. At 11 mg/m^3 (day 90), most endpoints in BAL were similar to the control groups. MWCNT were cleared from lung tissue over time. Histopathology revealed an increased cellularity in the bronchio-alveolar region with focal septal thickening and focal septal collagen deposition at 241 mg/m^3. Despite a concentration-dependent increase of Co in lung tissue, determinations in the remaining tissues were unobtrusive. In this study, LC_0 was greater than 241 mg/m^3 [Bayer MaterialSciencs AG, 2011; Baytubes: Acute toxicity: inhalation.001][29].

The second study was conducted according to OECD test guideline (No. 403) under GLP. Male and female SD rats were exposed for 6 hrs by inhalation **Hanwha CM-100** at concentrations of 0, 0.15, 0.39 and 1.33 mg/m3. They were observed for 14 days after inhalation, and were sacrificed for gross pathology. No toxic effects were detected in clinical observation or necropsy. LC0 was greater than 1.33 mg/m3 in this study [MKE. Korea, 2011; Hanwha CM-100: Acute toxicity: inhalation.001][30].

Dermal

Two reliable reports are available as for acute dermal toxicity of MWCNT. Details of the study are as follows.

The first study was conducted in accordance with OECD guideline (No. 402) under GLP. Male and female SD rats (5 animals/sex/dose) were treated by dermal application under semi-occlusive dressing for 24 hrs with 2,000 mg/kg bw of **Graphistrength C100**. They were observed for 14 days, thereafter necropsied. Black coloration of the skin was observed in all the treated animals, and this coloration masked the evaluation of cutaneous reactions. Crust formation was observed in one male and two females after day 11. LD0 was greater than 2,000 mg/kg bw [Arkema, Graphistrength C100: Acute toxicity: dermal.001][31.]

The second study was conducted in accordance with OECD guideline (No. 402) under GLP. Male and female SD rats (5 females/dose) were treated by dermal application under semi-occlusive dressing for 24 hrs with 2,000 mg/kg bw of **Hanwha CM-100**. MWCNT was dispersed in DPPC solution (5.5 mM D-(+)-glucose + 0.6 mg/ml Bovine serum albumin + 0.01 mg/kg DPPC). After 14-days observation period, they were sacrificed for gross pathology. No toxic effects were observed. LD0 was greater than 2,000 mg/kg bw [MKE. Korea, 2011; Hanwha CM-100: Acute toxicity: dermal.001][32.]

As additional information, Baytubes was reported to be no toxicity up to 2000 mg/kg-bw by OECD TG 402 test[5].

Oral

Four reliable reports are available as for acute oral toxicity of MWCNT. Details of the study are as follows.

The first study was conducted in accordance with OECD test guideline (No. 423) under GLP. Three female SD rats were administered orally by gavage with **Nikkiso MWCNT** in 5% acacia aqueous solution at 200 mg/kg bw. As the bulk density of Nikkiso MWCNT is extremely high level, the dose was achieved by separate four times treatment of 50 mg/kg bw in one hour interval due to limitation of the concentration with preferable dispersion state and dosing volume by ethical reason. After administration of 200 mg/kg bw of MWCNT, no abnormality was found in the first 3 animals, and then additional 3 female rats were also given the same dose similarly. Six animals were observed for 14 days after administration, and sacrificed for necropsy. Black feces were found in all rats on the following day of administration and in one rat on the day 2, but disappeared thereafter. No deaths and no toxicological effects were observed. LD0 of MWCNT was greater than 200 mg/kg bw in female SD rats [Matsumoto, M. et al., 2012; Nikkiso MWCNT: Acute toxicity: oral.001][33].

The second study was also conducted in accordance with OECD test guideline (No.423) under GLP. Female SD rats (6 animals/dose) were given **Graphistrength C100** suspended in 0.5% methyl cellulose solution by gavage at dose of 300 and 2,000 mg/kg bw. They were observed for 14 days after administration, and then necropsied. No deaths occurred at either dose. The abnormalities found in clinical signs were hypoactivity, piloerection and dyspnea at 2,000 mg/kg bw. LD0 of MWCNT was greater than 2,000 mg/kg bw in female SD rats [Arkema; Graphistrength C100: Acute toxicity: oral.001][34].

The third study was also conducted in accordance with OECD test guideline (No. 423) under GLP. Female SD rats (6 animals) were orally administered with **Hanhwa CM-100** at a maximum dose of 300 mg/kg bw. MWCNT was dispersed in DPPC solution (5.5 mM D-(+)-glucose + 0.6 mg/ml Bovine serum albumin + 0.01 mg/kg DPPC). Single oral administration of MWCNT did not cause any signs of toxicity up to 300 mg/kg bw. LD0 was greater than 300 mg/kg bw [MKE. Korea, 2011; Hanhwa CM-100: Acute toxicity: oral.001][35].

The Fourth study was also conducted in accordance with OECD test guideline (No. 420). Female SD rats (4 animals) were orally administered with **Nanocyl NC7000** at a maximum dose of 100 mg/kg bw. MWCNT was dispersed in HPC solution (1% HPMC). Single oral administration of MWCNT did not cause any signs of toxicity up to 100 mg/kg bw within 24 hours. The granulomatous changes in the liver were observed. However, the absence of dose dependence questioned the toxicological relevance of this observation. [Nanocyl, 2008; Nanocyl NC7000: Acute toxicity: oral.001][36].

Besides, acute oral toxicity study was executed as a preliminary study of micronucleus assay using mice. In the study, male ICR mice were administered by gavage with **Nikkiso MWCNT** twice in interval of 24 hrs at 5, 10 and 20 mg/kg bw. 0.3% CMC was used as the vehicle of the test substance. Twice doses of MWCNT caused no abnormality up to 20 mg/kg bw in male mice [Nikkiso MWCNT: Acute toxicity: oral.002][37.]

As additional information, Baytubes was reported to be no toxicity up to 5000 mg/kg-bw by OECD TG 423 test[5].

Other Routes of Exposure

There are several reports with single administration by intratracheal instillation or pharyngeal aspiration on rats or mice. These studies were chiefly focussed on acute pulmonary toxicity and its persistency.

For example, **Nikkiso MWCNT** dispersed in distilled water including 0.05% Triton X, intratracheally instilled at doses of 0.2 or 1.0 mg/head to male Wistar rats, induced pulmonary inflammation evidenced by

BALF examinations and histopathology. A transient neutrophil infiltration was observed in the low dose group, while presence of small granulomatous lesions and persistent neutrophil infiltration in the high dose group, which lasted until 6 months after instillation [Morimoto, Y. et al., 2012; Nikkiso MWCNT: Acute toxicity: other routes. 001][38].

A single intratracheal instillation with **Graphistrength C100** suspension in DME medium at doses of 10 or 100 μg/head to male Balb/C mice also induced pulmonary granulomatous inflammation that persisted at 6 months after instillation [Tabet, L. et al., 2011; Graphistrength C100: Acute toxicity: other routes. 001][39.]

Mitsui MWNT-7 treated with single intratracheal instillation also induced pulmonary inflammatory responses in rats of two experiments administered at 5 mg/head in the former [Wako, K, et al., 2010; Mitsui MWNT-7: Acute toxicity: other routes. 001][40] and the latter one up to 1.0 mg/kg bw [Kobayashi, N. et al., 2010; Mitsui MWNT-7: Acute toxicity: other routes. 002][41]. In the former experiment, MWCNT suspended in artificial lung surfactant (ALS) with grinding in agate ball mill induced pulmonary inflammatory responses, but MWCNT without grinding did not induce remarkable responses, indicating that the amount of agglomerates in the suspension is an important factor affecting the pulmonary toxicity of MWCNT.

Mitsui MWNT-7 suspended in dispersion medium was treated by single pharyngeal aspiration to male C57BL mice at doses of 10, 20, 40 or 80 μg/head. Treatment caused pulmonary inflammation in a dose-dependent manner and peaked at 7 days post exposure. Histopathology revealed rapid development of pulmonary fibrosis, and granulomatous inflammation persisted up to 56 days post exposure [Porter, D.W. et al., 2010; Mitsui MWNT-7: Acute toxicity: other routes. 003][42]. In the same experiment, the average thickness of connective tissue in the alveolar septa was increased by 45% in the 40 μg and 73% in the 80 μg exposure group versus control group. This indicated MWCNTs have the potential to produce a progressive fibrotic response in the alveolar tissues in the lung [Mercer, R.R. et al., 2011; Mitsui MWNT-7: Acute toxicity: other routes. 004][43]. Furthermore, from the lung tissues obtained on 7 days and 56 days post exposure in the same experiment, 4 specific genes were identified as candidate lung cancer prognostic genes [Pacurari, M. et al., 2011; Mitsui MWNT-7: Acute toxicity: other routes. 005][44]. In the other study on single pharyngeal aspiration with Mitsui MWNT-7 (40 μg/head) to mice, pulmonary inflammatory responses were also observed up to 28 days post exposure, and serum acute phase proteins with immune function including complement C3, apoprotein A-I and A-II, and alpha2-microglobulin were increased. MWCNT exposure induced measurable systemic markers but lacked specificity to distinguish from other pulmonary exposure [Erdely, A. et al., 2011; Mitsui MWNT-7: Acute toxicity: other routes. 006][45].

One report is available for acute effects after single intraperitoneal injection. **Mitsui MWNT-7** was incubated in Gambles solution (simulated biological fluids) for 0 weeks or 10 weeks, and were filtered and resuspended in 0.5% bovine serum albumin. A presumed mass of 50 μg was injected into the peritoneal cavities of female C57BL/6 mice. They were sacrificed at 24 hrs or 7 days post injection, and the peritoneal cavities were washed and inflammatory responses were examined in the lavage fluids collected. The MWCNT incubated in Gambles solution for 0 weeks induced an acute inflammatory response at 24 hrs post exposure that did not subside by 7 days after injection, and also induced a strong fibrotic response at 7 days. On the other hand, the MWCNT incubated in Gambles solution for 10 weeks was less pathogenic in mice, inducing reduced inflammatory and fibrotic responses compared to those of 0 weeks. Since the test substance lost 30% of its original mass when incubated *in vitro* in Gambles solution for the first three weeks and decrease in the proportion of long fibers observed in electron microscopy, the loss of pathogenicity was considered to be accompanied with the loss of mass and fiber shortening *in vitro* [Osmond-McLeod, M.J. et al., 2011; Mitsui MWNT-7: Acute toxicity: other routes. 007][46].

Studies in Humans

Inhalation

No information is available.

Dermal

No information is available.

Oral

No information is available.

Other Routes of Exposure

No information is available.

Conclusion

In the two reliable acute inhalation studies with MWCNT in rats, no deaths occurred up to the concentration of 241 mg/m^3 in spite of pulmonary toxicity. LC_0 in inhalation exposure was over 241 mg/m^3. From the two reliable acute dermal toxicity studies in rats, dermal LD_0 was greater than 2,000 mg/kg bw. For acute oral toxicity, there were three reliable studies using rats. No deaths occurred up to the highest dose tested in each study, and LD_0 values of MWCNTs ranged from over 200 mg/kg bw to over 2,000 mg/kg bw. Clinical signs observed included black feces, hypoactivity, piloerection and dyspnea. Besides, single administration experiments of MWCNT by intratracheal instillation, pharyngeal aspiration or intraperitoneal injection were performed, and pulmonary or intraperitoneal inflammatory responses were confirmed.

IRRITATION

Skin Irritation

Studies in Animals

In vivo Studies

Two reliable study reports are available. Details of the study are as follows.

The first study was conducted in accordance with OECD test guideline (No. 404). 0.5 g of two types of MWCNT **(Nikkiso MWCNT** and **Mitsui MWNT-7**) in olive oil was applied occlusively for 4 hrs on a shaved back skin of three male NZW rabbits. Score of skin irritation was evaluated at 1, 24, 48 and 72 hrs after removal of patch, and primary irritation index (P.I.I) was calculated as a mean of scores at 24-72 hrs. In case of **Nikkiso MWCNT**, very slight erythema was observed in all three rabbits at 24 and 48 hrs which disappeared at 72 hrs. The value of P.I.I was 0.6, which showed that the test substance was slightly irritating. While, in case of Mitsui **MWNT-7**, no cutaneous reaction occurred. The value of P.I.I was 0.0,

which leads to the conclusion that the test substance was not irritating [Ema, M. et al. (2011); Nikkiso MWCNT: Skin irritation / corrosion.001; Mitsui MWNT-7: Skin irritation / corrosion.001][47].

The second study was also conducted in accordance with OECD test guideline (No. 404). Three male NZW rabbits were tested with 0.5 g of **Graphistrength C100** on shaved one-side flank for 4 hrs. The observation period was 72hrs after removal of dressing, but observation continued until day 8. Due to blackish coloration of skin, scoring of erythema could not be done during 72 hrs observation period, but very slight erythema was observed in one rabbit on days 4 and 5. Oedema was not observed in all rabbits during the observation period. Taking the possible erythema masked by coloration into account, the maximal mean values of over 24, 48 and 72 hrs for erythema could be 0.3, 1.0 and 1.3, respectively, which leads to the conclusion that the test substance was slightly irritating [Arkema; Graphistrength C100: Skin irritation / corrosion.001][48].

As additional information, Nanocyl NC7000 was reported to be neither irritative nor corrosive by OECD TG 431 test[9].

As another additional information, Baytubes was reported not to be irritant by OECD TG 404 test[5].

In vitro Studies

Though reliability of the study was not assigned due to insufficient documentation, there are two reports as for *in vitro* dermal corrosion assay (OECD TG 431). According to them, both types of MWCNT (**Nanocyl NC 7000** and **Baytubes**) were concluded as not corrosive [Nanocyl, 2011; Nanocyl NC 7000: Skin irritation / corrosion.001[49]; Baytubes: Skin irritation / corrosion.001[50]].

Studies in Humans

In vivo Studies

No information on humans is available.

In vitro Studies

No information on humans is available.

Eye Irritation

Studies in Animals

In vivo Studies

Two reliable study reports are available. Details of the study are as follows.

The first study was conducted similar to OECD test guideline (No. 405) using two types of MWCNTs. 0.1 mL each of 0.25% **(Nikkiso MWCNT**) or 1.0% (**Mitsui MWNT-7**) MWCNTs suspension in a minimum amount of olive oil was instilled in the conjunctivae of the eye of three male NZW rabbits for around one second, and the eye was rinsed 1 hour later. At 1, 24, 48 and 72 hrs, the eyes were observed and scored. In case of **Nikkiso MWCNT**, redness of conjunctivae (score=1) was observed in all 3 rabbits at 1 hr after instillation, but recovered within 24 hrs. No other changes were observed in any rabbits during the observation period. As a result, it was concluded that the 0.25% suspension was slightly irritating. On the other hand, **Mitsui MWVT-7** did not produce any irritant response on rabbit eyes. It was concluded

that the 1.0% suspension was not irritating [Ema, M. et al., 2011; Nikkiso MWCNT: Eye irritation.001; Mitsui MWNT-7: Eye irritation.001][47].

The second study was conducted in accordance with OECD test guideline (No. 405) under GLP. Three NZW rabbits were treated with 0.1 g of **Graphistrength C100** into the conjunctival sac of left eye of each animal. Approximately 24 hrs after instillation, eyes were rinsed with saline. Ocular reactions were observed and scored at 1, 24, 48 and 72 hrs after rinsing, and the reversibility of the ocular response was then daily confirmed until up to day 21. As a result, irritant effects on the eyes were observed in all three animals. The mean scores calculated for each animal over 24, 48 and 72 hrs were 3.0, 2.0 and 2.7 for chemosis, 2.3, 2.0 and 2.7 for redness of the conjunctivae, 0.3, 0.7 and 1.0 for iris lesions, and 3.0, 1.7, 2.3 for corneal opacity. A part of the eye lesions did not disappear until the end of the study. Thus, the test substance was irritating [Arkema; Graphistrength C100: Eye irritation.001] [51].

As additional information, Baytubes was reported to be slight irritant by OECD TG 405 test[5].

In vitro Studies

One reliable study report is available. A BCOP assay was conducted in accordance with OECD test guideline (No. 437) under GLP. **Graphistrength C100** was applied to bovine fresh cornea and incubated in vitro for 4 hrs. After completion of incubation, residual test substance was observed on corneas treated with MWCNT. The in vitro irritancy score (IVIS) in MWCNT treated group was 0.2. Since IVIS value of the test substance was below 55.1, MWCNT was concluded as not corrosive or not severe irritant [Arkema; Graphistrength C100: Eye irritation.002] [52].

Studies in Humans

No information is available.

Respiratory Tract Irritation

Studies in Animals

Acute (see section 4.1.2) and repeated dose toxicity (section 4.1.5) studies demonstrated that the respiratory tract is the target organ after inhalation exposure to MWCNT characterized by an inflammatory response.

Studies in Humans

No information is available.

Conclusion

In the skin irritation tests with rabbits, three types of MWCNTs showed no or slight irritancy. In the eye irritation test with rabbits, diluted suspension of two types of MWCNTs caused no or slight irritant response. However, the third test substance (**Graphistrenth C100**) showed marked ocular responses with poor reversibility. One of the reasons of the difference between these results was thought to be the difference of test substance. A dry powder of MWCNT of the latter study and diluted suspensions in oil for the former two studies. In *in vitro* BCOP method, **Graphistrenth C100** showed very small irritancy score, and was not classified as severe irritant, both indicating that the eye irritation didn't result from a cytotoxic effect but was secondary to an abrasive (mechanical) action. MWCNT are irritating to the respiratory tract.

Sensitisation

Studies in Animals

Skin

Two reliable study reports are available. Details of the study are as follows.

In the first study, Buehler test with guinea pig was performed using two types of MWCNTs. This study was conducted in accordance with OECD test guideline (No.406). 0.4 g of MWCNTs (**Nikisso MWCNT** and **Mitsui MWNT-7**) in olive oil was epicutaneously applied to male Hartley guinea pigs once a week, three times in total (day 0, 7 and 14) in 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 hrs. Both types of MWCNT-treatment groups gave a negative result (20 animals/group), while 0.1% DNCB as a positive control showed 100% positive response (10 animals/group) [Ema, M. et al., 2011; Nikisso MWCNT: Skin sensitisation.001; MWNT-7: Skin sensitisation.002][47].

In the second study, Local Lymph Node Assay (LLNA) using mice was performed. This study was conducted in accordance with OECD test guideline (No.429) under GLP. Female CBA mice (4 animals/dose) were applied to ear skin with 0.25, 0.5, 1.0 and 2.5% of **Graphistrength C100** in propylene glycol, and presence or absence of lymphoproliferation was examined as an indication. Twenty five percent hexyl cinnamic aldehyde (HCA) as a positive control showed a significant lymphoproliferative response, while any MWCNT treated group did not cause a significant proliferative response. Thus, the test substance was concluded as not sensitizing [Arkema; Graphistrength C100: Skin sensitisation.001][53].

As additional information, Baytubes was reported to be no sensitizing effect by OECD TG 406 test[5].

Respiratory Tract

No information is available.

Studies in Humans

Skin

No information is available.

Respiratory Tract

No information is available.

Conclusion

MWCNT was concluded as not skin sensitising based on negative results of both two types in guinea pig Buehler method and another type in murine LLNA method.

REPEATED DOSE TOXICITY

Studies in Animals

Inhalation

Here, studies which the exposure period was longer than 2 weeks with referring to NOAEL/LOAEL were selected. Consequently, five reliable study reports are available. Details of the study are as follows.

The first study was 2-week inhalation study with 4-week post exposure recovery in rats in accordance with OECD test guideline (No. 412) under GLP. Male and female Fischer 344 rats (10 animals/sex/dose) were exposed by whole body inhalation with aerosol of **Mitsui MWNT-7 (**purity: 99.8%) at concentrations of 0.2, 1.0 and 5.0 mg/m^3 for 6 hrs/day, 5 days/week for 2 weeks. The highest dose was determined from the preliminary study[53]. Recovery test groups for 4-weeks post exposure was set as satellite animals. Examinations were almost fully performed, but histopathology was restricted to the respiratory organs and associated lymph nodes, liver and kidneys. Biochemical and cytological analyses of bronchio-alveolar lavage fluid (BALF) were additionally examined. Treatment-related effects were seen only in the respiratory tract. In BALF examinations at the end of exposure period, the numbers of neutrophils, percentages of bi- and multi-nucleated alveolar macrophages, levels of alkaline phosphatise (ALP) activity, and concentrations of total protein and albumin were elevated in the rats exposed to 1 mg/m^3 and higher. After 4-weeks recovery period, the values of BALF parameters tended to remain elevated. Histopathology revealed MWCNTs deposition remained mostly in the lung (all treatment groups), goblet cell hyperplasia in nasal cavity and nasopharynx (1 and 5 mg/m^3), and granulomatous changes in the lung (5 mg/m^3) at the end of exposure period. After 4- week recovery period, goblet cell hyperplasia was regressed, but granulomatous changes were slightly aggravated. Based on the inflammatory changes in BALF examinations and findings in histopathology, NOAEL in this study was determined as 0.2 mg/m^3 [Umeda, Y. et al., 2013; Kasai, T. et al., 2013; Mitsui MWNT-7: Repeated dose toxicity: inhalation.001][54, 55].

The second study was 4-week inhalation study with 3-months post exposure recovery in rats. Male Wistar rats were exposed by whole body inhalation with aerosol of **Nikkiso MWCNT** (purity: >98%) dispersed in Triton X-100 solution at a mean concentration of 0.37 mg/m^3 for 6 hrs/day, 5 days/week for 4 weeks. After completion of exposure period, the rats were sacrificed at 3 days, 1 month and 3 months post exposure. This study was mainly focussed on pulmonary toxicity of MWCNT. Pulmonary toxicity was evaluated by biochemical and cytological examinations on BALF, chemokine analysis of lung tissue and BALF, and lung histopathology. As a consequence, in MWCNT exposed group, the lung weight and neutrophil cell count in BAL increased only at the third day after the end of exposure period. Histopathology of the lung revealed no inflammatory changes but only to a slight extent of alveolar macrophages phagocysed MWCNT [Morimoto, Y. et al., 2012[56]; Oyabu, T. et al., 2011[57]; Nikkiso MWCNT: Repeated dose toxicity: inhalation.001]. Thus, grade of the pulmonary toxicity was minimal and no obvious inflammatory changes recognized. Nakanishi et al. presented a view in a risk assessment of carbon nanotubes that the concentration of 0.37 mg/m^3 was considered as NOAEL in this study [Nakanishi, J. et al., 2011][58].

The third study was 13-week inhalation study with rats in accordance with OECD test guideline (No.413). Male and female Wistar rats (10 animals/sex/dose) were exposed by nose/head only inhalation with **Nanocyl NC7000** (purity: 90%) at concentrations of 0, 0.1, 0.5 and 2.5 mg/m^3 on 6 hrs/day, 5 days/week for 13 weeks. Examinations were fully performed. In haematology, total WBC count increased accompanying with increase of neutrophil differential ratio and decrease of lymphocyte differential ratio in males and females exposed to 2.5 mg/m^3. Relative lung weights were increased in males and females exposed to 0.5 mg/m^3 and higher. Histopathology revealed pronounced multifocal granuromatous

inflammation, diffuse histiocytic and neutrophilic inflammation, and intra-alveolar lipoproteinosis were observed in the lung and lung-associated lymph nodes at 0.5 and 2.5 mg/m^3 in both sexes. In these groups, inflammatory changes were also observed in nasal cavity. At 0.1 mg/m^3, there was still minimal granuromatous inflammation in the lung and lung-associated lymph nodes. NOAEL was therefore not established in this study [Ma-Hock, L. et al., 2009; Nanocyl NC7000: Repeated dose toxicity: inhalation.001][59].

The fourth study was 13-week inhalation study with rats in accordance with OECD test guideline (No. 413). Male and female Wistar rats were exposed by nose only inhalation with **Baytubes** at concentrations of 0, 0.1, 0.4, 1.5 and 6 mg/m^3 on 6 hrs/day, 5 days/week for 13 weeks. Examinations were fully performed. Additionally, BALF examination was conducted to evaluate pulmonary toxicity. As a result, treatment-related effects were limited to respiratory organs. The lung and lung-associated lymph nodes (LALN) weights were significantly increased at concentrations of 0.4 mg/m^3 and higher. Elevation of polymorphonuclear neutrophils and soluble collagen in BALF were observed at concentrations of 0.4 mg/m^3 and higher. Histopathology revealed principal treatment-related lesions in the upper respiratory tract (goblet cell hyperplasia and/or metaplasia, eosinophilic globules, focal turbinate remodeling) and lower respiratory tract (inflammation changes in the bronchio-alveolar region, increased interstitial collagen staining) at concentrations of 0.4 mg/m^3 and above. Granulomatous changes and broncheo-alveolar hyperplasia occurred at 6 mg/m^3. All endpoints examined were unremarkable at 0.1 mg/m^3. NOAEL was determined as 0.1 mg/m^3 in this study [Pauluhn, J., 2010a; 2010b; Baytubes: Repeated dose toxicity: inhalation: 001][60, 61] .

The fifth study was also 13-week inhalation study with rats in accordance with OECD test guideline (No. 413). Male and female F344/DuCrlCrlj rats were exposed by whole body inhalation with **Hodogaya (former Mitsui) MWNT-7** at concentrations of 0, 0.2, 1 and 5 mg/m^3 on 6 hrs/day, 5 days/week for 13 weeks. The aerosol was generated by newly developed dry type generator. Examinations were fully performed and additionally, BALF examination and lung burden examination (amounts of MWCNTs in the lung) were conducted. A few MWCNTs were observed in the subpleural area and diaphragm. Moreover, the lung burden of MWCNTs demonstrated that incidences and severity of toxicity depended on exposure concentration, duration and retention. The lowest-observed-adverse-effect level (LOAEL) was estimated to be 0.2 mg/m^3 with the endpoints of granulomatous changes and BALF parameters in the present study. [Kasai, T. et al., 20114; Hodogaya (Mitsui) MWNT-7 : Repeated dose toxicity: inhalation: 004][62].

Further, two studies of five-days inhalation were conducted for **Graphistrength C100** and **Hanwha CM-95.**

Male and female Wistar rats were exposed by nose only inhalation with **Graphistrength C100** at concentrations of 0.066, 0.26, and 1.3 mg/m3 on 6 hrs/day for 5 days. A slight increase in neutrophil count was observed at 1.30 mg/m3 after the 5-day treatment, which disappeared after the 4-week recovery period. GGT levels were statistically significantly increased at 1.30 mg/m3 but were normal after the recovery and protein values were statistically significantly increased at 0.26 and 1.30 mg/m3 after exposure and after the 4-week recovery period. Macrophage infiltration of the lung (grade 2) was observed at 1.30 mg/m3in 3 males and 3 females after the 5-day exposure and in 4 males and 4 females after the recovery period. Hypertrophy of the bronchial and bronchiolar cells was observed at 1.30 mg/m3in 4 males (3 grade 1 and 1 grade 2) and 2 females (1 grade 1 and 1 grade 2) after the 5-day exposure and in 2 males and 2 females (all grade 1) after the recovery period. No treatment-related microscopic findings were observed in the other organs examined. [Arkema; Graphistrength C100: Repeated dose toxicity: inhalation. 001][63]

Male Sprague-Dawley rats were exposed by whole body inhalation with **Hanwha CM-95** at concentrations of 0, 0.16, 0.34, and 0.94 mg/m3 on 6 hrs/day for 5 days. The animals exhibited no significant body weight changes, abnormal clinical signs, or mortality during the experiment. Although the H2O2 Concentration in BAL did not show any statistical significance with any of the MWCNT concentrations, there were some increasing trends. At one month after the 5-day exposure, the H2O2 concentration exhibited an increasing trend, although there was no statistical significance. The MWCNT-exposed lungs showed that the MWCNTs were deposited in the alveolar epithelium and the alveolar macrophages after the 5-day inhalation exposure. The deposition of the MWCNTs also persisted even after 30 days postexposure, although the deposition amount was significantly reduced. [Hanwha CM-100: Repeated dose toxicity: inhalation. 001][64]

Dermal

No information is available.

Oral

One reliable study report is available. Details of the study are as follows.

A 28-day oral repeated dose toxicity study in rats was reported. This study was identified as a key study because it was conducted in accordance with the OECD test guideline (No. 407). Details of the study [Matsumoto, M. et al., 2012; Nikkiso MWCNT: Repeated dose toxicity: oral.001][33] are as follows. Crl:CD (SD) rats (6 animals/sex/dose) were given **Nikkiso MWCNT (** purity: >98%) at doses of 0, 0.5, 5 and 50 mg/kg bw/day (vehicle: 5% Acacia aqueous solution) once daily by gavage. As recovery test groups, 6 animals/sex/dose of 0 and 50 mg/kg bw/day were set as satellite animals. The administration and recovery period was 28 days and 14 days, respectively. As described in the section of acute oral toxicity, 50 mg/kg bw was the highest dose practically prepared in this study because of extremely higher level of the bulk density. General and detailed clinical observations, measurement of body weight and food consumption, and hematology, clinical chemistry, urinalysis, measurement of organ weights, gross pathology, and histopathology were examined.

There were no treatment-related changes in the any groups of both sexes except for black feces in all treatment groups and greyish green or dark green coloured contents in large intestine observed in males and females at 5 mg/kg/day or more. But these changes were considered not toxic effects. Therefore, no abnormality was found up to the highest dose tested in 28-day oral repeated dose study of MWCNT in rats. NOAEL in this study was determined to be 50 mg/kg bw/day in both sexes.

As additional information, Nanocyl NC7000 was reported to be no toxicity up to 0.5 mg/kg-day by OECD TG 420 test[9].

Studies in Humans

Inhalation

No information is available.

Dermal

No information is available.

Oral

No information is available.

Conclusion

There are four reliable studies on repeated toxicity studies of MWCNTs via inhalation route. Rats were used in all the studies. The exposure period and the test concentrations ranged from 2 weeks to 13 weeks, and from 0.1 mg/m^3 to 6 mg/m^3, respectively. From the results of the 2-week and two 13-week inhalation studies in accordance with OECD test guideline, conducted with full protocol, no systemic toxicity was observed except for haematological changes descried below. Toxic effects were restricted to the respiratory tract and lung-associated lymph nodes. Inflammatory changes were commonly observed mainly in the nasal cavity and lungs evidenced by BALF examinations and histopathology. These inflammatory effects were concentration related, and granulomatous changes in the lungs were recognized at higher concentration. In one 13-week inhalation study, increases in the number of WBC and neutrophil differential ratio in blood were found, which was considered as an effect reflecting pulmonary inflammation. The lowest NOAEL determined among four studies was 0.1 mg/m^3 in a 13-week inhalation study with **Baytube**, while, in another 13-week study with **Nanocyl NC7000** pulmonary inflammatory changes were still observed at the respective concentration, representing NOAEL as below 0.1 mg/m^3. At present, effects of repeated dose toxicity studies of MWCNT by inhalation were restricted to the respiratory organs, and no obvious systemic effects confirmed. NOAEL of MWCNTs via inhalation was considered as c.a. 0.1 mg/m^3.

There is only one reliable study on repeated toxicity study of MWCNT via oral route. This study was 28-day oral repeated dose toxicity study in rats. The highest dose was set as 50 mg/kg/day due to technically applicable maximum dose. No toxic effects were detected up to the highest dose, and NOAEL of MWCNT via oral route was considered as over 50 mg/kg/day.

Mutagenicity

Studies in Animals

In vitro Studies

Bacterial mutation test

Four reliable study reports are available. Details of the study were as follows.

The first test was conducted in accordance with OECD test guideline No.471. Two types of MWCNTs (**Nikkiso MWCNT** and **Mitsui MWNT-7**) were incubated with five strains of bacteria (TA1535, TA1537, TA98, TA100 and E.coli. WP2uvrA) at several concentrations up to 100 μg/plate with and without S9 mix. As a result, both types of MWCNT were negative at any concentrations for all strains, regardless of presence or absence of metabolic activation. The positive controls (sodium azide, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 9-aminoacridine or 2-aminoanthracene) showed expected levels of mutagenicity [Ema, M. et al., 2012; Nikkiso MWCNT: Genetic toxicity in vitro.001; Mitsui MWNT-7: Genetic toxicity in vitro.001][65].

The second study was conducted in accordance with OECD test guideline No. 471. **Graphistrength C100** was incubated with five strains of bacteria (Salmonella typhimurium: TA1535, TA1537, TA98, TA100 and TA 102) at concentrations of 15.6, 31.3, 62.5, 125 and 500 μg/plate with and without S9 mix. The test substance was not mutagenic in all strains with and without S9, while positive controls produced effective results [Arkema; Graphistrength C100: Genetic toxicity in vitro:001][66].

The third study was conducted in accordance with OECD test guideline No. 471. Baytubes was incubated with five strains of bacteria (Salmonella typhimurium: TA1535, TA1537, TA98, TA100 and TA 102) at concentrations up to 5,000 μg/plate with and without S9 mix. The test substance was negative in any conditions [Wirnitzer, U. et al., 2009; Baytubes: Genetic toxicity in vitro.001][67].

The fourth study was conducted in accordance with OECD test guideline No. 471. **Hanwha CM-95** was incubated with five strains of bacteria (TA1535, TA1537, TA98, TA100 and E.coli.WP2uvrA) at several concentrations up to 333 μg/plate with and without S9 mix. The test substance was not mutagenic in all strains with and without S9, while positive controls produced effective results [MKE. Korea, 2011; Kim, J. S. et al., 2011; Hanwha CM-100: Genetic toxicity in vitro.001][68].

As additional information, Nanocyl NC7000 was reported not to be mutagenic up to highest possible dose of 2000 μ g / plate by OECD TG 471 test[9].

Mammalian cell gene mutation test

Two reliable study reports are available. Details of the study are as follows.

The first study was conducted in accordance with OECD test guideline No. 476. **Graphistrength C100** was cultured with L5178Y mouse lymphoma cells at concentrations up to 20 μg/mL for 3 hrs or 24 hrs with and without S9 mix. Under these conditions, although slight to marked precipitations were observed, no noteworthy increase in mutation frequency in comparison to vehicle control was noted. The positive controls (methylmethanesulfonate and cyclophosphamide) showed the expected performances. The test substance was concluded as not mutagenic [Arkema; Graphistrength C100: Genetic toxicity in vitro.002][69].

The second study was conducted in accordance with OECD test guideline No. 476. **Mitsui MWNT-7** was cultured with Chinese hamster lung cells (CHL/IU) for 48 hrs at concentrations ranging from 6.3 to 100 μg/mL without S9 mix. Then the cells were rinsed with PBS and incubated in a normal medium for 6 days. After 6 days incubation, the cells were treated with trypsin, and the cells were transferred to culture dishes containing 6-thioguanine (6-TG) for mutation selection. Cell viability and the number of 6-TG resistant colonies were measured and the mutation frequency was expressed as the number of 6-TG resistant cells per 10^6 cells corrected by the cell viability. Consequently, mutation rate per 10^6 cells did not increase up to the concentrations of 100 μg/mL of MWCNT in spite of dose-dependent decrease in cell viability. Ethyl methanesulphonate, the positive control, increased markedly in mutation rate. The test substance was concluded as not mutagenic [Asakura, M. et al., 2010; Mitsui MWNT-7: Genetic toxicity in vitro.002][70].

As additional information, Baytubes was reported negative by OECD TG 476 test[5].

Chromosomal aberration test

Five reliable study reports are available. Details of the study are as follows.

The first study was conducted in accordance with OECD test guideline No. 473. Two types of MWCNTs (**Nikkiso MWCNT** and **Mitsui N-MWNT**) were cultured with Chinese hamster lung fibroblast cell line (CHL/IU) at concentrations up to 100 μg/mL with and without S9 mix. Consequently, increases of structural chromosomal aberrations were not observed in any dose of both types of MWCNT with and without S9 mix. However, frequency of cells with numerical chromosomal aberrations were found slightly higher in **Nikkiso MWCNT** and strongly in **Mitsui N-MWNT** at 100 μg/mL without S9. Positive controls (mitomycin C, benzo(a)pyrene) gave expected performances [Ema, M. et al., 2012; Nikkiso MWCNT: Genetic toxicity in vitro.002; Mitsui N-MWNT: Genetic toxicity in vitro.003][71].

The second study was conducted in accordance with OECD test guideline N0. 473. **Graphistrength C100** was cultured with human lymphocytes at concentrations up to 50 μg/mL with and without S9 mix. Since precipitation occurred at concentrations of 25μg/mL or more, observation of chromosomal aberrations was conducted up to the highest concentration of 12.5 μg/mL. As a consequence, no significant increase in frequency of cells with structural aberrations was noted in any concentration tested with and without S9 mix [Arkema; Graphistrength C100: Genetic toxicity in vitro.003][72].

The third study was conducted in accordance with OECD guideline No. 473. **Mitsui MWNT-7** was cultured with CHL/IU for 24 hrs at concentrations ranging from 1.3 to 80 μg/mL or 48 hrs at concentrations ranging from 0.078 to 5.0 μg/mL without S9 mix. Consequently, structural chromosomal aberrations were not observed. However, significant increased number of cells with numerical aberrations (polyploidy) was observed at concentrations of 5 μg/mL or more in 24 hrs treatment and at concentrations of 1.3 and 5.0 μg/mL in 48 hrs treatment [Asakura, M., 2010; Mitsui MWNT-7: Genetic toxicity in vitro:004][70]

The fourth study was conducted in accordance with OECD test guideline No. 473. **Baytubes** was cultured with V79 Chinese hamster lung fibroblasts at concentrations of 2.5, 5 and 10 μg/mL with and without S9 mix. Under these conditions, the test substance showed neither cytotoxicity nor chromosomal aberrations [Wirnitzer, U. et al., 2009; Baytubes: Genetic toxicity in vitro:002][67]

The fifth study was also conducted in accordance with OECD guideline No. 473 under GLP. **Hanwha CM-95** was cultured with Chinese hamster ovarian fibroblasts (CHO-K1) at concentrations up to 6.25 μg/mL for 6hrs and 24 hrs without S9, or at concentrations up to 25 μg/mL for 6 hrs. The test substance showed no chromosomal aberrations in any conditions [MKE. Korea, 2011; Kim, J.S. et al., 2011; Hanwha CM-100: Genetic toxicity in vitro.002][68]

Others

A study for in vitro mammalian cell micronucleus test was reported. **Mitsui MWNT-7** was incubated with CHL/IU cells at concentrations up to 5.0 μg/mL for 48 hrs without metabolic activation. The number of micronucleated cells in 2,000 intact interphase cells was counted as indication. The number of bi- or multinucleated cells were also counted. As a result, MWCNT significantly increased the numbers of bi-nucletaed and multi- nucleated cells without micronucleus induction [Asakura, M. et al., 2010; Mitsui MWNT-7: Genetic toxicity in vitro.005][70]

In vivo Studies

Two reliable study reports are available. Details of the study are as follows.

In the first study, micronucleus assay using mice was performed in accordance with OECD test guideline No. 474. Male and female ICR mice (6 animals/dose) were administered orally by gavage with either one of two types of MWCNTs (**Nikkiso MWCNT** and **Mitsui MWNT-7**) at doses of 0, 5, 10 and 20 mg/kg bw/day, once daily for 2 consecutive days. At 24 hrs after the second administration, they were sacrificed and the bone marrow was collected from the femur. The bone marrow cells were prepared and 2,000 immature erythrocytes were observed to count the rate of the micronucleated polychromatic erythrocytes (MNPCEs). Mitomycin C was used as a positive control. As a result, the incidence of MNPCEs in the either type of MWCNT-treatment groups was not different from that in the negative control group, while the incidence in the positive control was significantly increased [Ema, M. et al., 2012; Nikkiso MWCNT: Genetic toxicity in vivo.001; Mitsui MWNT-7: Genetic toxicity in vivo.001][65]

In the second study, micronucleus assay using mice was also conducted in accordance with OECD test guideline No. 474. Male ICR mice (6 animals/dose) were treated intraperitoneally with **Hanwha CM-95** at

doses of 0 (vehicle: DPPC), 12.5, 25 and 50 mg/kg bw. At 24 hrs after treatment, mice were sacrificed, and bone marrow cells were collected from the femurs. The cells with micronucleus were counted on 2,000 polychromatic erythrocytes. Consequently, MNPCE ratio was not increased by treatment with MWCNT, while significantly increased with mitomycin C-treatment [MKE. Korea, 2011; Kim, J.S. et al., 2011; Hanwha CM-100: Genetic toxicity. in vivo.001][65]n the third study, male SD rats (10 animals/dose) were exposed by whole body inhalation with **Hanwha CM-95** (alternative product of CM-100) at concentrations of 0, 0.16, 0.34 or 0.94 mg/m^3 for 6 hrs/day for 5 days. The rats were killed at the end of exposure period and 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. As a result, OTM was significantly elevated in the group exposed to the highest concentration (148 percent 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. The MWCNT exposed by inhalation at high concentration caused a statistically significant increase in lung DNA damage [Kim, J.S. et al., 2012; Hanwha CM-100: Genetic toxicity in vivo:002][73].

Studies in Humans

No information is available.

Conclusion

In *in vitro* mutagenicity tests for bacterial as well as mammalian gene cell mutations, MWCNTs tested showed all negative. In *in vitro* chromosomal aberration assays with mammalian cells, three of five types of MWCNT gave negative results as for not only structural but also numerical aberrations. However, the other two types of MWCNTs **(Nikkiso MWCNT** and **Mitsui MWNT-7**) caused numerical aberrations (polyploidy) at high concentration in spite of no structural aberrations. With regard to possible mechanism of induction of polyploidy in chromosomal aberration test with MWCNT, Asakura et al. (2010) suggested that MWCNTs has a property of interfering physically with biological process during cytokinesis, but not directly with DNA.

On the contrary, in *in vivo* studies with **Hanwha CM-95**, a micronucleus assays using mice was negative by intraperitoneal route. The Comet assay using lung cells isolated from rats exposed by inhalation to **Hanwha CM-95** showed an evidence of DNA damage in exposed lung tissues.

Thus, there are some positive results with *in vitro* clastogenicity and *in vivo* DNA damage in genotoxicity of MWCNTs in spite of the negative results in most of the studies, leading to the conclusion at present that genotoxic potential of MWCNTs need more investigations.

Carcinogenicity

In vitro Studies

No information is available.

In vivo Studies in Animals

Inhalation

No information is available.

Dermal

No information is available.

Oral

No information is available.

Other routes of exposure

Five study reports are available. Details of the study are as follows.

The first study was conducted as two-year follow up study after single intraperitoneal injection. Male Wistar rats (50 animals/group) were treated intraperitoneally with **Nanocyl NC7000** with or without structural defects (MWCNT+: with defects; MWCNT-: without defects). The doses applied were 2 or 20 mg/rat for MWCNT+ and 20 mg/rat only for MWCNT-. The vehicles control (phosphate buffered saline) and 2 mg of crosidolite asbestos treatment groups (26 animals/group) were set simultaneously. 24 months post exposure, they were subjected to necropsy and the incidence of mesotheliomas and other tumours in the peritoneal cavity was investigated. As a consequence, crosidolite induced clear carcinogenic response (34.6% animals with mesothelioma vs. 3.8% in vehicle control), while MWCNT with or without structural defects did not induce mesothelioma in this bioassay (incidence of 2 and 20 mg/rat for MWCNT+, 20 mg/rat for MWCNT-; 4, 0 and 6%, respectively). The incidence of tumours other than mesotheliomas was not significantly increased across the groups [Muller, J. et al., 2009; Nanocyl NC7000: Carcinogenicity.001][74].

The second study was conducted as a long-term follow up study after single intraperitoneal injection using p53 heterozygous (p53+/-) mice. Male p53 hetrozygous mice were treated intraperitoneally with **Mitsui MWNT-7** at dose of 3 mg/head (corresponds to $1x10^9$ particles/head). The vehicle control (0.5% CMC supplemented with 1.0% Tween 80) and the crosidolite asbestos ($1x10^{10}$ particles corresponding to 3 mg/head) treatment group were treated similarly. They were maintained up to 25 weeks after injection, and observed for tumours in intraperitoneal cavity. Consequently, the overall incidence of mesothelioma found in the MWCNT group on day 84 were 14/16 (87.5%, 11 found as cause of death, 3 as incidental) in MWCNT and 14/18 (77.8%, 8 found as cause of death, 6 as incidental including 3 terminated at week 25) in the crosidolite group. Neither tumour induction nor interim death occurred in the control group. It was considered that MWCNT injected intraperitoneally in p53(+/-) mice carcinogenesis model induced mesothelioma, probably due to its resemblance to asbestos in size, shape and biopersistency [Takagi, A. et al., 2008; Mitsui MWNT-7: Carcinogenicity.001][75]. However, although mesothelioma development in this study was observed to be a cause of death, peritoneal adhesion (and fibrous thickening) which causes constriction of the ilius, was also considered to contribute to the observed mortality. A foreign body response to MWCNTs was also observed, so that granulomas were evident, with fibrosis also being a feature of the response.

The third was conducted as an additional dose response study of the second study with p53+/- mice. Male p53+/- mice (20 animals/dose) were treated by single intraperitoneal injection with **Mitsui MWNT-7** at doses of 0 (vehicle: 0.5% methyl cellulose supplemented with 1.0% Tween 80), 3, 30 or 300 μg/head (corresponding to $1x10^6$, $1x10^7$, $1x10^8$ particles/head), and observed for up to one year after injection. As a result, the cumulative incidence of peritoneal mesotheliomas was increased in a dose-dependent manner (5/20, 17/20 and 19/20). The severity of peritoneal adhesion and granuloma formation were dose-

dependent and minimal in the lowest dose group. All mice in the lowest dose group that survived until the terminal kill had microscopic atypical mesothelial hyperplasia considered as a precursor lesion of mesothelioma [Takagi, A. et al., 2012; Mitsui MWCNT-7: Carcinogenicity.002][76].

Mitsui MWNT-7 was also studied in male F344 rats. MWCNT was administrated to 7 rats by a single intrascrotal injection at 1 mg/kg bw, and observed up to 54 weeks. Six animals died or became moribund due to intraperitoneally disseminated mesothelioma with bloody ascites after 37-40 weeks. Peritoneal mesothelium was generally hypertrophic, and numerous nodular or papillary lesions of mesothelioma and mesothelial hyperplasia were developed. While mesothelioid cells were predominant in relatively early stage tumors, advanced stage mesotheliomas were constituted by 2 portions occupied by mesothelioid cells on the surface and spindle-shaped sarcomatous cells in the depth. In the latter, the histological transition was apparently observed between these 2 portions. Mesotheliomas were invasive to adjacent organs and tissues, and frequently metastasized into the pleura. Only 1 rat survived for 52 weeks in the MWCNT-treated group, and similar findings except mesothelioma were observed. All 10 crocidolite-treated and 5 vehicle-treated rats survived for 52 weeks without any particular changes except deposition of asbestos in the former case [Sakamoto et al., 2009][77].

Male F344 rats were treated with 500 μg/mL suspensions of **Mitsui MWNT-7** and **Nikkiso MWCNT** five times over a 9-day period by intrapulmonary spraying. Multi-walled carbon nanotubes were found mainly in alveolar macrophages and mediastinal lymph nodes. Importantly, the fibers were also found in the cell pellets of the pleural cavity lavage, mostly in macrophages. Multi-walled carbon nanotube treatment induced hyperplastic proliferative lesions of the visceral mesothelium, with their proliferating cell nuclear antigen indices approximately 10-fold that of the vehicle control. The hyperplastic lesions were associated with inflammatory cell infiltration and inflammation-induced fibrotic lesions of the pleural tissues. The fibers were not found in the mesothelial proliferative lesions themselves. In the pleural cavity, abundant inflammatory cell infiltration, mainly composed of macrophages, was observed [Xu et al., 2012][78].

Studies in Humans

No information is available.

Conclusion

There are no studies of carcinogenicity of MWCNT via inhalation, dermal or oral route. However, its carcinogenic potential on induction of peritoneal mesothelioma was examined when administered intraperitoneally. Using p53 heterozygous mice, a sensitive model for tumorigenesis, it was demonstrated that a single intraperiotoneal injection with a certain MWCNT (**Mitsui MWNT-7**) induced the peritoneal mesothelioma in a dose-dependent manner. The author suggested its carcinogenic potential was probably due to its resemblance to asbestos. Subsequently, mesothelioma was also observed in rats exposed to Mitsui MWNT-7 or **Nikkiso MWCNT**. On the contrary, another type of MWCNT (**Nanocyl NC7000**) did not induce mesothelioma in rats after two year of single injection, while crosidolite, a positive control, succeeded to induce methothelioma at high incidence. Further studies will be needed for carcinogenicity of MWCNTs, particularly in consideration of differences among MWCNTs for both physicochemical and biological properties.

Toxicity for Reproduction

Studies in Animals

Effects on Fertility

No fertility studies reported. In repeated dose toxicity studies including inhalation studies for 2 weeks to 13 weeks and an oral 28-day study, no effects were seen in the reproductive organs of both sexes in rats. This might suggest no effect on fertility in inhalation and oral treatment with MWCNTs.

Developmental Toxicity

Two reliable study reports are available. Details of the study are as follows.

The first study was conducted according to the method similar to OECD test guideline No. 414 except for less number of animals used. Pregnant female SD rats (12 inseminated females per dose) were orally administered by gavage with MWCNTs (**Hanwha CM-95**; alternative product of CM-100) for 14 days starting from gestational day 6(GD6) until GD19. The doses given were 0(vehicle: 1% CMC solution), 40, 200 or 1,000 mg/kg bw/day. All dams were sacrificed on GD20, and the fetuses were morphologically examined for external, visceral or skeletal anomalies. As a result, the only change observed as maternal toxicity was a decrease in thymus weight observed in 1,000 mg/kg bw/day group. Morphological examinations of the fetuses demonstrated no significant difference in incidences of anomalies between the groups. NOAEL of MWCNTs was concluded to be 200 mg/kg bw/day for maternal toxicity and 1,000 mg/kg bw/day for embryo-fetal development. No teratogenic effect was observed [Lim, J-H. et al., 2011a; 2011b; Hanwha CM-100: Developmental toxicity / teratogenicity.001][79].

The second study was performed to examine a teratogenic potential of MWCNTs compulsory injected into the body prenatally. MWCNTs (**Mitsui MWNT-7**) were suspended in 2% CMC solution and given intraperitoneally (2, 3, 4 or 5 mg/kg bw) or intratracheally (3, 4 or 5 mg/kg bw) to pregnant ICR mice on GD9. They were sacrificed on GD18, and the fetuses removed from the uterus were examined for external and skeletal anomalies. In the intraperitoneal study, various types of malformations were observed in all MWCNT-treated groups, while such malformations were observed in groups given 4 or 5 mg/kg bw, but not in that treated with 3 mg/kg bw in the intratracheal study. In either study, the number of litters having fetuses with external and that having fetuses with skeletal malformations were both increased in a dose dependent manner [Fujitani, T. et al., 2012; Mitsui MWNT-7:Developmental toxicity / teratogenicity.001][80].

Studies in Humans

Effects on Fertility

No information is available.

Developmental Toxicity

No information is available.

Conclusion

No fertility studies reported. However, no effect on fertility might be considered in rats via oral and inhalation routes, considering that no effects on the reproductive organs in repeated dose toxicity studies in these routes. With regard to developmental toxicity, there are two available studies. One is a typical teratogenicity study administered by gavage during organogenesis period in rats. The other is a special

study to examine the teratogenic potential in mice of MWCNTs administered by unusual routes. The former results demonstrated that MWCNTs given orally during organogenesis did not induce either fetotoxic or teratogenic effect up to the maximum dose of 1,000 mg/kg bw/day, where minimal maternal toxicity was observed. On the other hand, the latter study revealed developmental effects in the offspring when the dams were treated with a single injection with another type of MWCNT either via intraperitoneal or intratracheal route. Although the contradictory results between both studies can not be compared directly due to large differences of the test conditions including the test materials, the former result have to be considered in line with the poor oral absorption of MWCNT (see section 4.1.1). The problem remaining with the latter study, suggesting that a certain type of MWCNT might exhibit a potential teratogenic effect in next generation neonates, is the use of no physiological routes of exposure for humans. Considering no information for effects on fertility, and the low relevance of the data available on the developmental toxicity, further studies will be needed for toxicity to reproduction of MWCNTs.

Adequate screening-level data, especially genotoxicity, carcinogenicity and toxicity to reproduction, are not necessary available to characterize the human health hazard for the purposes of the OECD HPV Chemicals Program.

REFERENCES

1 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Biodegradation test of Nikkiso MWCNT. CERI 15605.

2 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Biodegradation test of Nikkiso MWCNT. CERI 15621.

3 Arkema, WPMN Graphistrength C100 Dossier 5.2.1 Graphistrength C100 Biodegradation TG 301F

4 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Biodegradation test of Nikkiso MWCNT. CERI 15622

5 Information from Bayer MaterialScience, MWCNT Dossier

6 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Short-term toxicity to fish of Nikkiso MWCNT. CERI 95399.

7 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 203) Short-term toxicity to fish.001]

8 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Long term toxicity test to fish of Nikkiso MWCNT.MCM A100701.

9 Information from Nanocyl, MWCNT Dossier

10 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Daphnia sp. Acute immobilisation test of Nikkiso MWCNT. CERI 95398.

11 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 202) Short-term toxicity to aquatic invertebrates.001]

12 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Algae growth inhibitation test of Nikkiso MWCNT. CERI 95397.

13 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 201)Toxicity to aquatic algae and cyanobacteria.001]

14 AIST, National Institute of Advanced Industrial Science and Technology, 2011. Long-term toxicity test to aquatic invertebrates of Nikkiso MWCNT. MCM A100701

15 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG211) Long-term toxicity to aquatic invertebrates.001]

16 AIST, National Institute of Advanced Industrial Science and Technology, 2011. [Nikkiso MWCNT T(TG201) oxicity to aquatic algae and cyanobacteria.001] CERI 95397

17 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG201) Toxicity to aquatic algae and cyanobacteria.002]

18 AIST, National Institute of Advanced Industrial Science and Technology, 2011. [Nikkiso MWCNT (TG 209) Effect on activated sludge at WWTP.001], CERI 95936

19 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 ([Graphistrength C100 (TG 209) Effect on activated sludge at WWTP]

20 AIST, National Institute of Advanced Industrial Science and Technology, 2011. [Nikkiso MWCNT (TG 216) Toxicity to soil microorganisms.001], MCM A100703

21 Arkema, WPMN Dossier [Graphistrength C100 Xenopus laevi micronucleus assay (ISO21427-1)]

22 Oyabu, T. et al. (2011) Biopersistence of inhaled MWCNT in rat lungs in a 4-week well-characterized exposure. Inhalation Toxicol., 23(13), 784-791

23 Taquahashi Y et al., Improved dispersion method of multi-wall carbon nanotube for inhalation toxicity studies of experimental animals. J Toxicol Sci. 2013;38(4):619-28.

24 Ohnishi M et al., Novel method using hybrid markers: development of an approach for pulmonary measurement of multi-walled carbon nanotubes. J Occup Med Toxicol. 2013, 25;8(1):30

25 Aiso, S. et al. (2011) Translocation of intratracheally instilled Multiwalled Carbon Nanotubes to lung-associated lymph nodes in rats. Industr. Health, 49, 215-220.

26 Jacobsen, NR. et al. (2013) WP 7: Toxicokinetics and tissue distribution of MNs for specification of organs at risk for genotoxicity testing. Deliverable 7: Identification of target organs and biodistribution including ADME parameters. http://www.nanogenotox.eu/files/PDF/DELIVRABLES2/deliverable7__biodistribution.pdf.

27 Georgin, D. et al. (2009) Preparation of 14C-labeled Multi-walled carbon nanotubes for biodistribution investigations. J. Amer. Chem. Soc., 131, 14658-14659.

28 Jani, P., Halbert, G. W., Langridge, J., Florence, A. T.: The uptake and translocation of latex nanospheres and microspheres after oral administration to rats“, J. Pharm. Pharmacol. 41, p. 809-812 (1989)

29 Bayer MaterialScience, WPMN Baytubes Dossier; Baytubes: Acute toxicity: inhalation.001

30 MKE. Korea, 2011; Acute inhalation toxicity study of MWCNT in rats

31 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 402) Acute toxicity: dermal.001]

32 MKE. Korea, 2011; Acute dermal toxicity study of MWCNT in rats

33 Matsumoto, M. et al. (2012) No toxicological effects on acute and repeated oral gavage doses of single-wall or multi-wall carbon nanotube in rats. J. Toxicol. Sci., 37(3), 346-474.

34 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 423) Acute toxicity: oral.001]

35 MKE. Korea, 2011; Acute oral toxicity study of MWCNT in rats

36 Nanocyl, REACH Dossier DISS-b281d1a0-c6d8-5dcf-e044-00144f67d031/AGGR-110ae1d5-073b-4d0f-a2ab-f3c0f8efe56d_DISS-b281d1a0-c6d8-5dcf-e044-00144f67d031.html#AGGR-110ae1d5-073b-4d0f-a2ab-f3c0f8efe56d ([Supporting oral acute toxicity 003]

37 AIST 2010, Ina Research ZT 10127, Micronucleus assay of Multiwalled Carbon Nanotube B using mice

38 Morimoto, Y. et al. (2012) Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation. Nanotoxicology. 6(6):587-599.Nanotoxicology, Early Online, 1-15.

39 Tabet, L. et al. (2011) Coating carbon nanotubes with a polystylene based polymer profiles against pulmonary toxicity. Particle and Fibre Toxicol., 8, 3-16.

40Wako, K. et al. (2010) Effects of preparation methods for multi-walled carbon nanotube (MWCNT) suspensions on MWCNT induced rat pulmonary toxicity. J. Toxicol. Sci., 35(4), 437-446.

41 Kobayashi, N. et al. (2010) Biological response and morphological asessement of individually dispersed multi-wall carbon nanotubes in the lung after intratracheal instillation in rats. Toxicology, 276, 143-153.

42 Porter, D.W. et al. (2010) Mouse pulmonary dose- and time course-responses induced by exposure to multi-walled carbon anotubes. Toxicology, 269, 136-147.

43 Mercer, R.R. et al. (2011) Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes. Particle and Fibre toxicol., 8, 21-32.

44 Pacurari, M. et al. (2011) Multi-walled carbon nanotube-induced gene expression in the mouse lung: Association with lung pathology. Toxicol. Appl. Pharmacol., doi:10.1016/j.taap,2011.05.012.

45 Erdely, A. et al. (2011) Identification of systemic markers from a pulmonary carbon nanotube exposure. J. Occup. Environ. Med., 53(6), S80-S86.

46 Osmond-McLeod, M.J. et al. (2011) Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres. Particle and Fibre Toxicol., 8, 15-33.

47 Ema, M. et al. (2011) Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotube. Regul. Toxicol. Pharmacol., 61, 276-281.

48 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 404) Skin irritation / corrosion.001]

49 Nanocyl, WPMN Nanocyl NC7000 Dossier; Nanocyl NC 7000: Skin irritation / corrosion.001; Baytubes: Skin irritation / corrosion.001

50 Bayer MaterialScience, WPMN Baytubes Dossier: Baytubes: Skin irritation / corrosion.001

51 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 405) Eye irritation. 001]

52 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 437) Eye irritation. 002]

53 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 429) Skin sensitisation.001]

54 Umeda, Y. et al. (2013) Two-week toxicity of multi-walled carbon nanotubes by whole-body inhalation exposure in rats. J. Toxicol., Pathol., 26(2), 131-140.

55 Kasai, T. et al. (2013) Development of a new multi-walled carbon nanotube (MWCNT) aerosol generation and exposure system and confirmation of suitability for conducting a single-exposure inhalation study of MWCNT in rats. Nanotoxicology, Early Online, 1-10 8, 169-178.

56 Morimoto Y. et al. (2012) Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation. Nanotoxicology, 6 (6):587-599

57 Oyabu T. et al. (2011) Biopersistence of inhaled MWCNT in rat lungs in a 4-week well-characterized exposure. Inhalation Toxicology, 23 (13) 784-791

58 Nakanishi, J. (ed) (2011) Risk Assessment of Manufactured Nanomaterials: Carbon Nanotubes (CNT). Final report issued on August 17, 2011. NEDO Project (P06401) "Research and Development Nanoparticle Characterization Methods"

59 Ma-Hock, L. et al. (2009) Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. Toxicol. Sci., 112, 468-481.

60 Pauluhn, J. (2010) Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: Toxic effects are determined by density of agglomerate structures, not fibrillar structures. Toxico. Sci., 113, 226-242

61 Pauluhn, J. (2010) Multi-walled carbon nanotubes (Baytubes): Approach for derivation of occupational exposure limit. Regul. Toxicol. Phamacol., 57, 78-89.

62 Kasai, T. et al. (2014) Thirteen-week study of toxicity of fiber-like multi-walled carbon nanotubes with whole-body inhalation exposure in rats. Nanotox (will be publish in 2014) Nanotoxicology, Eary Online, 1-10. DOI: 10.3109/17435390. 2014. 933903.

63 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249, [Graphistrength C100: Repeated dose toxicity inhalation 001]

64 Kim JS et al.,Persistent DNA damage measured by Cometassay of Sprague-Dawley rat lung cells after five days of inhalation exposure and 1 month post-exposure to dispersed multi-wall carbon nanotubes (MWCNTs) generated by new MWCNT aerosol generation system, Toxicol. Sci., 128 (2): 439-448, (2012)

65 Ema, M. et al. (2012) Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of in vitro and in vivo assays. Regul. Toxicol. Pharmacol., YRTPH 2716, 11 April. 2012

66 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 471) Genetic toxicity in vitro.001].

67 Wirnitzer, U. et al. (2009) Studies on the in vitro genotoxicity of Baytubes agglomerates of engineered multi-walled carbon nanotubes (MWCNT). Toxicol. Lett., 186(3), 16

68 Kim, J.S. et al. (2011) Aspect ratio has no effect on genotoxicity of multiwall carbon nanotubes. Arch. Toxicol., 85, 775-786.

69 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 476) Genetic toxicity in vitro.002]

70 Asakura, M. et al. (2010) Genotoxicity and cytotoxicity of multi-wall carbon nanotubes in cultured Chinese hamster lung cells in comparison with Chrysotile A fibres. J. Occup. Health, 52, 155-166.

71 Ema, M. et al., 2012; Nikkiso MWCNT: Genetic toxicity in vitro.002; Mitsui N-MWNT: Genetic toxicity in vitro.003

72 Arkema, REACH Dossier DISS-abda73cd-a2cd-3801-e044-00144f67d249 [Graphistrength C100 (TG 473) Genetic toxicity in vitro.003]

73 Kim, J.S. et al. (2012) Persistent DNA damage measured by Comet assay of Sprague-Dawley rat lung cells after five days of inhalation exposure and 1 month post-exposure to dispersed multi-wall carbon nanotubes (MWCNTs) generated by new MWCNT aerosol generation system. Toxicol. Sci., 128(2), 439-448.

74 Muller, J. et al. (2009) Absence of carcinogenic response to multiwall carbon nanotubes in a two year bioassay in the periotoneal cavity of the rat. Toxicol. Sci., 110(2), 442-448.

75 Takagi, A, et al. (2008) Induction of methotelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. J. Toxicol. Sci., 33(1), 105-116.

76 Takagi, A. et al. (2012) Dose-dependent methothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice. Cancer Sci., 103(8), 1440-1444.

77 Sakamoto Y. et al., Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. J Toxicol Sci. 2009 Feb;34(1):65-76.

78 Xu J. et al., Multi-walled carbon nanotubes translocate into the pleural cavity and induce visceral mesothelial proliferation in rats, Cancer Sci. 2012; 103(12):2045-50.

79 Lim, J-H. et al., 2011a; 2011b; Hanwha CM-100: Developmental toxicity / teratogenicity.001

80 Fujitani, T. et al., 2012; Mitsui MWNT-7:Developmental toxicity / teratogenicity.001