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**ENVIRONMENT DIRECTORATE
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THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**DOSSIER ON MULTIWALLED CARBON NANOTUBES (MWCNT)
- PART 3 -**

**Series on the Safety of Manufactured Nanomaterials
No. 49**

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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 49

**DOSSIER ON MULTIWALLED CARBON NANOTUBES (MWCNT)
- PART 3 -**



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

**Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2015**

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No. 47, *Dossier on Nanoclays (2015)*

No. 48, *Dossier on Fullerenes (2015)*

No. 50, *Dossier on Single-Walled Carbon Nanotubes (SWCNTs) (2015)*

No. 51, *Dossier on Silicon dioxide (2015)*

No. 52, *Dossier on Zinc oxide (2015)*

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PREAMBLE

In November 2007, OECD's Working Party on Manufactured Nanomaterials (WPMN) launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The outcomes of the Testing Programme were intended to assess the applicability of the existing **test guidelines**¹ to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions. Understanding the properties of NMs is crucial to choose appropriate strategies for hazard identification, risk assessment or risk management measures. The Testing Programme involved delegations from OECD member countries, some non-member economies and other stakeholders. The broad international representation, from a range of delegations enabled the programme to pool expertise and resources without which this programme would not have been possible.

Before launching the Testing Programme, the WPMN first identified a broad list of possible nanomaterials, and the list was later adjusted to a final selection of eleven MNs for testing². This list comprised: i) fullerenes (C60); ii) single-walled carbon nanotubes (SWCNTs); iii) multi-walled carbon nanotubes (MWCNTs); iv) silver nanoparticles; v) titanium dioxide; vi) cerium oxide; vii) zinc oxide; viii) silicon dioxide; ix) dendrimers; x) nanoclays; and xi) gold nanoparticles. One fundamental criterion for selecting these materials was that they should be either in commercial use at the time or expected to be in the near future. At the same time, other considerations were also given attention, such as the production volume of the materials, the likely availability of such materials for testing and the existing information that would readily be available on the materials.

It was also agreed that 59 endpoints would be addressed³ for each material corresponding to the following categories: i) nanomaterial information/ identification; ii) physical-chemical properties and material characterisation; iii) environmental fate; iv) toxicological and eco-toxicological effects; v) environmental toxicology; and vi) mammalian toxicology. These endpoints were judged to be most important based largely on the general experience of testing chemicals, while taking into account the potentially different or new properties of nanomaterials. It is worth noticing that it was not expected that testing for all of the listed endpoints would be necessary for each of the selected MNs.

To assist with the Testing Programme, the WPMN developed two documents: i) a *Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials* [ENV/JM/MONO(2009)21]; and ii) *Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme* (Guidance Manual) in 2009, which was subsequently updated in 2010

¹ The OECD Test Guidelines are a collection of internationally agreed test methods used by government, industry and independent laboratories. They are used to determine the safety of chemicals.

<http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm>

² Originally Iron nanoparticles, Aluminium, Carbon black, and Polystyrene were suggested but later withdrawn and replaced by gold nanoparticles.

³ As specified in the Guidance Manual, "address" includes the term "completed" which provides that all dossiers will contain the identified endpoint information. Note that for some endpoints (for example, solubility) it is specified that the endpoint must be "completed". In such instances "completed" means that all Dossiers will be providing this endpoint information.

[ENV/JM/MONO(2009)20/REV]⁴. The objective of this *Guidance Manual* was to guide sponsors⁵ in the testing of the materials while ensuring that the information collected was reliable, accurate, consistent and therefore also comparable. The *Guidance Manual* addressed a whole range of issues including the organisation of the work.

The *Guidance Manual* contains detailed information on the selected endpoints for testing and recommendations on sample preparation and dosimetry.

The *Guidance Manual* also described the development of *Dossier Development Plans* (DDPs). These plans were prepared by Lead sponsors, Co-sponsors together with contributors to describe the specific plan for the testing of each nanomaterial including when and where the testing will be undertaken and by whom. The DDPs also included information on the materials to be tested as well as information on issues such as sample preparation and dosimetry. Each of the DDPs was prepared and reviewed by the WPMN before testing work began.

Based on the lessons learned during the Testing Programme, the WPMN also developed *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials* [ENV/JM/MONO(2012)40]. This latter document is an update of an earlier text first published in 2010.

The work on OECD's Testing Programme was completed by the end of 2013. In June 2014 the WPMN agreed that for each nanomaterial the dataset would be published in IUCLID printed format^{6 7}. The document will include the protocols and methods to allow their wider use (regulators and researchers).

The dataset in this document has been declassified and made publicly available and it is expected regulators and researchers will wish to use it. Due to a broad dissemination of the data and the exploratory setting in which they were developed there are a number of limitations in using the data of which potential users should be aware. The programme focused on answering scientific questions in the field of the OECD test guidelines but not to provide conclusions on the hazard or risk of the materials selected. The absence of data for some endpoints may be a gap for some endpoints but for other end points there may not if the data was not considered necessary. Although the programme ensured a broad participation of many stakeholders it was not intended to arrive at any pre-defined regulatory datasets requirements or risk assessment decisions. It was recognised from the beginning that the exploratory nature of the work would require subsequent follow-up work for example to review the specific needs that may arise when performing risk assessment of nanomaterials. In this context, the programme's ultimate goal, to add to the knowledge of the properties of nanomaterials, would form a cornerstone.

⁴ It is worth noting that while the *Guidance Manual for Sponsors* was primarily intended as a guide to WPMN's Testing Programme, it is also expected that it will be of value to anyone involved in testing NMs.

⁵ The *Guidance Manual* noted, for example, that there could be three levels of participation to the programme. Lead sponsors, who would assume responsibility for conducting or coordinating all of the testing, determined to be appropriate for each of the endpoints for a specific nanomaterial. In some cases, "joint lead" arrangements were developed. Co-sponsors conducted some of the testing determined to be appropriate and feasible to address the endpoints for a specific listed nanomaterial. Contributors provided test data, reference or testing materials or other relevant information to the lead and co-sponsors.

⁶ IUCLID is a software programme for the administration of data on chemical substances. Although it was originally developed to fulfill requirements in the EU for the evaluation and control of the risks of existing chemical substances, it is used by many others.

⁷ SIAR = SIDS Initial Assessment Report (SIDS = Screening Information Data Set)

FOREWORD

As part of its Programme on the Safety of Manufactured Nanomaterials, OECD launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The Testing Programme mainly aimed to assess the applicability of the existing test guidelines to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions.

This document presents the Dossier of the carbon nanomaterials: Single-Walled Carbon Nanotubes (SWCNT), Multiwalled Carbon Nanotubes (MWCNT) and Fullerenes (C60), which was prepared under the leadership of Japan and the United States. This nanomaterial has been tested for a number of endpoints for: i) Nanomaterials Information / Identification; ii) Physical-Chemical Properties; iii) Environmental Fate; iv) Environmental Toxicology; and v) Mammalian Toxicology. They have been analysed using OECD Guidelines for the Testing of Chemicals (TG)⁸. The data is presented in an IUCLID⁹ style format and includes the protocols and methods used (see Preamble).

Japan and the United States led the Testing Programme on carbon nanomaterials. This included the determination of the tests that were appropriate for carbon nanomaterials, performing a number of tests, as well as coordinating tests performed and inputs provided by other participating country and stakeholder, from Korea and the Business and Industry Advisory Committee to the OECD (BIAC).

Due to the large amount of information generated throughout the OECD Testing Programme on carbon nanomaterials, each dossier has been split into several parts, as follows:

- Single-Walled Carbon Nanotubes (SWCNT): 2 parts
- Multiwalled Carbon Nanotubes (MWCNT): 3 parts
- Fullerenes (C60): 2 parts

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

⁸ <http://www.oecd.org/env/testguidelines>

⁹ IUCLID is a software programme for the administration of data on chemical substances. It was originally developed to fulfil requirements in the EU for the evaluation and control of the risks of existing chemical substances. It is specifically relevant in the context of an international programme for the initial assessment of chemical substances.

ACKNOWLEDGMENTS

The OECD Secretariat and the WPMN is thankful to Japan and to the United States for leading the Testing Programme on Carbons, including Single-Walled Carbon Nanotubes (SWCNT), Multiwalled Carbon Nanotubes (MWCNT) and Fullerenes (C60). They are specifically grateful to Hiroyuki Hanawa from the Ministry of Economy, Trade and Industry of Japan, and to Philip Sayre from the Environment Protection Agency, USA. In addition, we appreciate the efforts made by other participating countries/ organisations, and in particular to those that coordinated the efforts within their respective delegations: Dr. Kyunghye CHOI from the Korean Ministry of Environment, as well as Dr. Daniel Bernard (Arkema, France) and Dr. Jacques Ragot (Bayer material Science, Germany) from the Business & Industry Advisory Committee to the OECD (BIAC).

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7.6 Genetic toxicity

7.6.1 Genetic toxicity in vitro

Endpoint study record: Baytubes: Genetic toxicity in vitro.001

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to OECD TG 471

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Bayer MaterialScience	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						
publication	Wirnitzer U, Herbold B, Voetz M, Ragot J.	2009	Studies on the in vitro genotoxicity of baytubesR, agglomerates of engineered multi- walled carbon- nanotubes (MWCNT)	Toxicology Letters, 186(3), 160-165, (2009)	BIAC (Wirnitzer et al.(4), Bayer HealthCare, Technology Services and MaterialScience)				

Materials and methods

Type of genotoxicity

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	

GLP compliance

no data

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Test material form

no data

Details on test material

Baytubes (Bayer MaterialScience, macroagglomerates of engineered MWCNTs)

Method**Species/strain****Species/strain** S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 102**Metabolic activation** with and without**Metabolic activation system** S9 mix**Test concentrations**

up to 5000 µg/plate

Details on test system and conditions

Baytubes were formulated in deionised water at 10 mg/ml and treated with ultrasound for 30 min at 25 degrees C. Particle size distribution was determined under the incubation conditions in the in vitro studies. In the Salmonella microsome (Ames) test (OECD TG 471) concentrations up to 5000 µg/plate were tested in Salmonella typhimurium (strains TA 1535, TA 100, TA 1537, TA 98 and TA 102) in the absence or presence of S9 mix.

Results and discussions**Test results****Species/strain** S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 102**Metabolic activation** with and without**Genotoxicity** negative**Cytotoxicity** no**Applicant's summary and conclusion****Interpretation of results**

negative

Conclusions

Under these conditions and in the concentration range tested there were no bacteriotoxic and no mutagenic effects.

Endpoint study record: Baytubes: Genetic toxicity in vitro.002

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to OECD TG 473

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Wirnitzer U, Herbold B, Voetz M, Ragot J.	2009	Studies on the in vitro genotoxicity of baytubesR, agglomerates of engineered multi-walled carbon-nanotubes (MWCNT)	Toxicology Letters, 186(3), 160-165, (2009)	BIAC (Wirnitzer et al.(4), Bayer HealthCare, Technology Services and MaterialScience)				
other: Information from Bayer MaterialScience	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Baytubes

Test material form

no data

Details on test material

Baytubes (Bayer MaterialScience, macroagglomerates of engineered MWCNTs)

Method**Species/strain****Species/strain** Chinese hamster lung fibroblasts (V79)**Metabolic activation** with and without**Test concentrations**

2.5, 5 and 10 µg/ml

Vehicle

water

Controls**Solvent / vehicle controls** yes (water)**Positive controls** yes**Positive control substance** cyclophosphamide
mitomycin C**Details on test system and conditions**

Baytubes were formulated in deionised water at 10 mg/ml and treated with ultrasound for 30 min at 25 degrees C. Particle size distribution was determined under the incubation conditions in the in vitro studies. In the chromosome aberration test V79 cells (OECD TG 473) were exposed in the absence or presence of S9 mix for 4 h to concentrations of 2.5, 5 and 10 µg/ml of Baytubes (visible from concentration of 5 µg/ml and higher). Harvest was 18 h after the beginning of the treatment. In addition, cells treated with 10 microg/ml were harvested 30 h after the beginning of the treatment. An additional experiment was

performed using continuous treatment at 2.5, 5 and 10 µg/ml for 18 h (no S9 mix) with subsequent harvest.

Results and discussions

Test results

Species/strain Chinese hamster lung fibroblasts (V79)

Metabolic activation with and without

Genotoxicity negative

Cytotoxicity no

Vehicle controls valid yes

Positive controls valid yes

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

Under the conditions and in the concentration range tested there were no cytotoxic and no clastogenic effects.

Endpoint study record: Baytubes: Genetic toxicity in vitro.003

Administrative Data

Purpose flag disregarded study

Study result type no data

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Summary data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Bayer MatetrialScience	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Results and discussions**Test results**

Genotoxicity negative

Applicant's summary and conclusion**Interpretation of results**

negative

Endpoint study record: Graphistrength C100: Genetic toxicity in vitro.001**Administrative Data**

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies GLP guideline study (OECD 471)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Arkema	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						
study report			REACH dossier	Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					

Materials and methods**Type of study**

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Test material form

no data

Details on test material

Test material was micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability.

Method**Species/strain**

Species/strain S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 102

Metabolic activation with and without

Metabolic activation system S9 mix

Test concentrations

15.6, 31.3, 62.5, 125, 500 µg/plate in the treatments with and without S9 mix.

Vehicle

ethanol

Controls

Solvent / vehicle controls yes (ethanol)

Positive controls yes

Positive control substance no data

Details on test system and conditions

The test item was conducted in two independent experiments, with and without a metabolic activation system, the S9 mix, prepared from a liver post-mitochondrial fraction (S9 fraction) of rats induced with Aroclor 1254. Both experiments were performed according to the direct plate incorporation method except for the second test with S9 mix, which was performed according to the preincubation method (60 minutes, 37°C). Five strains of bacteria Salmonella typhimurium: TA 1535, TA 1537, TA 98, TA 100 and TA 102 were used. Each strain was exposed to at least five dose-levels of the test item (three plates/dose-level).

After 48 to 72 hours of incubation at 37°C, the revertant colonies were scored. The evaluation of the toxicity was performed on the basis of the observation of the decrease in the number of revertant colonies and/or a thinning of the bacterial lawn. The test item Graphistrength C100 micronized was suspended in ethanol. The test item was found not soluble in the vehicles usually used for this type of assay. Consequently, a suspension was selected for the treatments. A homogenous suspension (to the naked eye) was obtained in ethanol at the concentration of 10 mg/mL. Since the test item was not soluble and non-toxic in the preliminary test, the highest dose-level was selected on the basis of the precipitate observed in the Petri plates, according to the criteria specified in the international guidelines.

Results and discussions

Test results

Species/strain S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 102

Metabolic activation with and without

Genotoxicity negative

Cytotoxicity yes

Vehicle controls valid yes

Positive controls valid yes

Any other information on results incl. tables

A moderate to marked precipitate was observed in the Petri plates when scoring the revertants at all dose-levels. In the first experiment without S9 mix, a marked toxicity was noted at the dose-level of 500 µg/plate in the five strains used. The test item did not induce any noteworthy increase in the number of revertants, either with or without S9 mix, in any of the five strains. The number of revertants for the vehicle and positive controls was as specified in the acceptance criteria. The study was therefore considered valid.

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

Graphistrength C100 micronized did not show any mutagenic activity in the bacterial reverse mutation test with Salmonella typhimurium.

Endpoint study record: Graphistrength C100: Genetic toxicity in vitro.002**Administrative Data**

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies GLP guideline study (OECD 476)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Arkema	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						
study report			REACH dossier	Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					

Materials and methods**Type of genotoxicity**

gene mutation

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Test material form

no data

Details on test material

Graphistrength C100 was micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability.

Method**Target gene**

tk locus

Species/strain

Species/strain mouse lymphoma L5178Y cells

Metabolic activation with and without

Metabolic activation system S9 mix

Test concentrations

The selected concentrations were 0.625, 1.25, 2.5, 5, 10 and 20µg/mL for both experiments, with and without S9 mix.

Vehicle

ethanol

Controls

Solvent / vehicle controls yes (ethanol)

Positive controls yes

Positive control substance methylmethanesulfonate

Remarks without metabolic activation

Solvent / vehicle controls yes (ethanol)

Positive controls yes

Positive control substance cyclophosphamide

Remarks with metabolic activation

Details on test system and conditions

The potential of Graphistrength C100 was evaluated in L5178Y mouse lymphoma cells. After a preliminary toxicity test, Graphistrength C100 micronized was tested in two independent experiments, with and without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9 fraction) of rats induced with Aroclor 1254. Approximately 0.5×10^6 (3-hour treatment) or 0.15×10^6 (24-hour treatment) cells/mL in 20 mL culture medium with 5% horse serum were exposed to the test or control items, in the presence or absence of S9 mix (final concentration of S9 fraction 2%), at 37°C. Cytotoxicity was measured by assessment of adjusted relative total growth (Adj. RTG) and relative suspension growth (Adj. RSG) as well as cloning efficiency following the expression time (CE2). The

number of mutant clones (differentiating small and large colonies) was checked after the expression of the mutant phenotype.

Any other information on materials and methods incl. tables

In the culture medium, the concentration of 50 µg/mL showed a moderate precipitate. At this dose-level, the pH and the osmolality values were comparable to those of the vehicle control culture. The cloning efficiencies CE2 and the mutation frequencies of the vehicle and positive controls were as specified in acceptance criteria. The study was therefore considered valid. Since the test item was non-toxic but poorly soluble, the choice of the highest dose-level was based on the level of precipitate, according to the criteria specified in the international guidelines.

Results and discussions

Test results

Species/strain mouse lymphoma L5178Y cells

Metabolic activation with and without

Genotoxicity negative

Cytotoxicity no

Vehicle controls valid yes

Positive controls valid yes

Any other information on results incl. tables

At the end of the treatment periods (3- or 24-hour treatments), a slight to marked precipitate was noted in the culture medium, at concentrations equal to or more 0.625µg/mL. Following the 3-hour treatment either with or without S9 mix as well as the 24-hour treatment without S9 mix, no toxicity was induced at any of the tested dose-levels as shown by the absence of any noteworthy decrease in the Adj. RTG. Following the 3-hour treatment either with or without S9 mix as well as the 24-hour treatment without S9 mix, no noteworthy increase in the mutation frequency, in comparison to the vehicle control was noted.

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

Graphistrength C100 micronized did not show any mutagenic activity in the mouse lymphoma assay.

Endpoint study record: Graphistrength C100: Genetic toxicity in vitro.003

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	GLP guideline study (OECD 473)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Arkema	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						
study report			REACH dossier	Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					

Materials and methods**Type of genotoxicity**

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Test material form

no data

Details on test material

Graphistrength C100 (micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability)

Method**Species/strain****Species/strain** other: human lymphocytes**Metabolic activation** with and without**Metabolic activation system** S9 mix**Test concentrations**

The treatment-concentrations were 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 µg/mL, for the first experiment, both with and without S9 mix, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 µg/mL, for the second experiment, both with and without S9 mix.

Vehicle

ethanol

Controls**Solvent / vehicle controls** yes (ethanol)**Positive controls** yes**Positive control substance** no data***Details on test system and conditions***

The potential of the test item Graphistrength C100 to induce chromosome aberrations was evaluated in cultured human lymphocytes. The test item was tested in two independent experiments, both with and without a liver metabolizing system (S9 mix), obtained from rats previously treated with Aroclor 1254. Treatment of cells with the test substance in the presence or absence of S9 resulted in similar numbers of aberrations compared to negative control (ethanol), while cell cultures treated with positive control substances resulted in significant elevation of aberrations. In the culture medium, the concentration of 50 µg/mL showed a marked precipitate. At this dose-level, the pH and the osmolality values were equivalent to those of the vehicle control culture. Since the test item was non-toxic but poorly soluble, the choice of the highest concentration was based on the level of precipitate, according to the criteria specified in the international guidelines.

Results and discussions**Test results****Species/strain** other: human lymphocytes**Metabolic activation** with and without**Genotoxicity** negative**Cytotoxicity** yes**Vehicle controls valid** yes**Positive controls valid** yes

Any other information on results incl. tables

A slight to marked precipitate was observed at the end of the treatment period, generally at concentrations equal to or more 12.5 µg/mL. Without S9 mix, following the 3-hour treatment, only a slight decrease in mitotic index was noted, without any clear evidence of a concentration relationship (up to 25% decrease). Following the 20-hour treatment, a slight decrease in mitotic index was noted at concentrations equal to or more 25 µg/mL (26-29% decrease). Following the 44-hour treatment, a slight decrease in mitotic index was noted at 50 µg/mL (24% decrease). The concentrations selected for metaphase analysis without S9 were 3.13, 6.25 and 12.5 µg/mL for the 3-hour treatment, the latter showing precipitate in the culture medium at the end of the treatment period, 3.13, 6.25 and 12.5 µg/mL for the 20-hour treatments, the latter showing precipitate in the culture medium at the end of the treatment period, and 25 µg/mL for the 44-hour treatment, this dose-level showing precipitate in the culture medium at the end of the treatment period. No significant increase in the frequency of cells with structural chromosomal aberrations was noted after 3-, 20- as well as 44-hour treatments. With S9, no noteworthy decrease in mitotic index was noted at the 20-hour harvest time in the first experiment. At the 20-hour harvest time in the second experiment, slight decreases in mitotic index were noted at concentrations equal to or more 12.5 µg/mL (26-31% decrease). No noteworthy decrease in mitotic index was noted at the 44-hour harvest time. The concentrations selected for metaphase analysis with S9 were 3.13, 6.25 and 12.5 µg/mL for the 20-hour harvest time in both experiments, the latter showing precipitate in the culture medium at the end of the treatment period, 12.5 µg/mL for the 44-hour harvest time, this dose-level showing precipitate in the culture medium at the end of the treatment period.

Overall remarks, attachments**Remarks on results including tables and figures**

No significant increase in the frequency of cells with structural chromosomal aberrations was noted in either experiments and at either harvest times.

Applicant's summary and conclusion**Interpretation of results**

negative

Conclusions

The study was considered valid. Graphistrength C100 micronized did not induce chromosome aberrations in cultured human lymphocytes.

Endpoint study record: Mitsui MWNT-7: Genetic toxicity in vitro.001**Administrative Data**

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to OEC TG 471

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J.	2012	Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of in vitro and in vivo assays	Regul Toxicol Pharmacol., 63(2):188-195, (2012)	Ina Research Inc.				

Materials and methods**Type of genotoxicity**

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Details on test material

- Name of test material (as cited in study report): MWNT-7 (Manufactured by Mitsui & Co.,Ltd. (Japan))
- Analytical purity: No data
- Impurities (identity and concentrations): Iron: 3600 ppm, Chromium: 14 ppm, Bismuth: 6 ppm, Nickel: 4 ppm
- Other BET surface area: 23 m²/g, Mean diameter: 70 nm

Method

Species/strain

Species/strain other: *S. typhimurium* TA100, TA98, TA1535, TA1537; and *E. coli* WP2uvrA

Metabolic activation with and without

Metabolic activation system S9 mix

Test concentrations

3.13, 6.25, 12.5, 25, 50, 100 µg/plate

Vehicle

- Vehicle(s)/solvent(s) used: 0.3% CMC-Na (sodium carboxymethyl cellulose)

Controls

Positive controls yes

Positive control substance sodium azide

Positive controls yes

Positive control substance sodium azide

other: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide

Solvent / vehicle controls yes (0.3% CMC-Na)

Positive controls yes

Positive control substance other: 9-aminoacridine
hydrochloride

Solvent / vehicle controls yes (0.3% CMC-Na)

Details on test system and conditions

Pre-incubation method with and without metabolic activation (S9 mix) was used.

Evaluation criteria

According to Ames et al., 1975 and US-FDA, 2000.

Results and discussions

Test results

Species/strain other: *S. typhimurium* TA100, TA98, TA1535, TA1537; and *E. coli* WP2uvrA

Metabolic with and without
activation

Genotoxicity negative

Cytotoxicity no

Vehicle yes
controls valid

Positive yes
controls valid

Any other information on results incl. tables

No strains showed the growth inhibition with or without metabolic activation. No mutation induction was found with or without metabolic activation in all concentrations and in all strains.

Table. Result of bacterial reverse mutation tests on MWNT-7

Test substance	concentration (µg/plate)	S9 mix	No. of revertant colonies/plate (Mean)					
			Base substitution type			Frameshift type		
			Ta100	TA1535	WP2uvrA		TA98	TA1537
MWNT-7	0	-	111	10	23		15	8
	3.13		118	12	15		14	9
	6.25		122	8	14		14	10
	12.5		123	10	19		16	14
	25		118	11	21		17	11
	50		121	8	18		19	12
	100		121	8	18		16	9
AF-2	0.01	+	798		98			
	0.1						513	
NaN3	0.5			554				
9AA	80							467
MWNT-7	0		126	7	16		31	12
	3.13		114	6	18		32	15
	6.25		135	9	23		27	13
	12.5		124	6	18		25	11
	25		124	8	26		29	12
	50		108	8	19		30	12
	100		134	11	18		27	12
2AA	0.5						407	
	1		971					
	2			221				243
	10				506			

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide

NaN3: Sodium azide

9AA: 9-aminoacridine hydrochloride

2AA: 2-Aminoanthracene

a: Precipitation

Overall remarks, attachments

Attached full study report

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

Neither type of the bacterial strains tested exerted mutagenicity with and without metabolic activation.

Endpoint study record: Mitsui MWNT-7: Genetic toxicity in vitro.002

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to OECD TG 473

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J.	2012	Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of in vitro and in vivo assays	Regul Toxicol Pharmacol., 63(2):188-195, (2012)	Ina Research Inc.				

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Details on test material

- Name of test material (as cited in study report): MWNT-7 (Manufactured by Mitsui & Co.,Ltd. (Japan))
- Analytical purity: No data
- Impurities (identity and concentrations): Iron: 3600 ppm, Chromium: 14 ppm, Bismuth: 6 ppm, Nickel: 4 ppm
- Other BET surface area: 23 m²/g, Mean diameter: 70 nm

Method**Species/strain****Species/strain** other: Chinese hamster lung fibroblast cell line CHL/IU**Metabolic activation** with and without**Metabolic activation system** S9 mix**Test concentrations**

6.25, 12.5, 25, 50 and 100 µg/mL

Vehicle

- Vehicle(s)/solvent(s) used: 0.3% CMC-Na (sodium carboxymethyl cellulose)

Controls**Solvent / vehicle controls** yes (0.3%CMC-Na)**Positive controls** yes**Positive control substance** mitomycin C**Positive controls** yes**Positive control substance** benzo(a)pyrene**Details on test system and conditions**

Treatment: Short-term treatment methods of 6hrs with and without metabolic activation and a continuous treatment method without metabolic activation were conducted.

Evaluation criteria

The clastogenic potential was judged as negative, equivocal, and positive if the incidence of cells showing any aberration was less than 5%, 5-10%, more than 10%, respectively.

Results and discussions**Test results**

Species/strain other: Chinese hamster lung fibroblast cell line CHL/IU

Metabolic activation with and without

Genotoxicity positive

Cytotoxicity no

Vehicle controls valid yes

Positive controls valid yes

Any other information on results incl. tables

Strong increase of numerical chromosome aberration was observed at 100 µg/mL of MWNT-7 without S9 mix. No increase of structural chromosome aberration was observed in any groups tested with and without metabolic activation.

Table. Results of chromosomal aberration test on MWNT-7 in a Chinese hamster lung cell line.

S9 mix (Duration of exposure)	Dose	No. of cells	% Structural aberrations		% Polyploid cells	Growth rate (%)
	(µg/mL)		+gap	-gap		
- (Short term) ^a	0	200	1.5	0.5	0.5	100
	6.25	200	0.5	0.5	1	110
	12.5	200	0	0	0.5	116
	25	200	0	0	1	101
	50	200	0	0	1.5	114
	100	200	0	0	25.5	104
	MMC 0.12	200	59.5	59.5	0	-
-+(Short term) ^a	0	200	0.5	0	0.5	100
	6.25	200	1	0.5	0.5	109
	12.5	200	0.5	0	1	111
	25	200	1.5	0.5	0	98
	50	200	1	0.5	0.5	104
	100	200	0.5	0.5	2.5	113
	B[a]P 30	200	41	40	0	-
- (Continuous) ^b	0	200	0	0	1	100
	6.25	200	0.5	0.5	0	101
	12.5	200	0.5	0	0.5	103
	25	200	0	0	1	101
	50	200	0	0	1.5	106
	100	200	0.5	0	20.5	97
	MMC 0.06	200	54	51	1	-

MMC: Mitomycin C

B[a]P: Benzo[a]pyrene

a Exposure time 6h, and recovery time was 18h

b Exposure time was 24h

Overall remarks, attachments

Attached full study report

Illustration (picture/graph)

Attachment Document: MWNT-7 Chromosome aberration Table.JPG / 37.1 KB with UUID: IUC5-341b0f92-5498-4782-8f07-2608494a7753 cannot be accessed

Results of preliminary cytotoxicity tests for chromosomal aberrations on multi-walled carbon nanotubes (MWCNTs).

Test substance	Concentration (µg/mL)	% Relative cell growth		
		Short-term exposure ^a		Continuous exposure ^b
		–S9 mix	+S9 mix	–S9 mix
N-MWCNTs	0	100	100	100
	0.78	101	96	103
	1.56	103	92	99
	3.13	95	94	106
	6.25	101	92	116
	12.5	104	96	117
	25	92	94	106
	50	104	95	114
	100	103	80	104
MWNT-7	0	100	100	100
	0.78	106	98	106
	1.56	105	102	104
	3.13	105	98	108
	6.25	106	102	113
	12.5	106	103	102
	25	98	102	106
	50	101	98	104
	100	95	94	102

^a Exposure time was 6 h, and recovery time was 18 h.

^b Exposure time was 24 h.

Results of chromosomal aberration tests on multi-walled carbon nanotubes (MWCNTs) in a Chinese hamster lung cell line.

Test substance	Concentration (µg/mL)	S9 mix (Duration of exposure)	No. of cells	% Structural aberrations		% Polyploid cells	% Relative cell growth
				+gap	-gap		
N-MWCNTs	0	– (Short-term) ^a	200	0	0	0.5	100
	12.5		200	0	0	0.5	108
	25		200	0	0	1.0	107
	50		200	1.5	1.0	1.0	98
	100		200	0.5	0.5	15.0	103
MMC	0.12		200	58.0	58.0	0	
N-MWCNTs	0	+ (Short-term) ^a	200	1.5	0	0.5	100
	12.5		200	1.0	0	0	94
	25		200	0	0	0	98
	50		200	1.0	0.5	1.5	89
	100		200	3.0	2.5	2.0	79
B[a]P	30		200	46.5	45.0	0.5	
N-MWCNTs	0	– (Continuous) ^b	200	1.0	1.0	0.5	100
	12.5		200	0.5	0.5	0.5	112
	25		200	1.0	1.0	1.0	104
	50		200	1.5	0.5	2.5	97
	100		200	1.0	0.5	6.5	96
MMC	0.06		200	54.0	53.0	0	
MWNT-7	0	– (Short-term) ^a	200	1.5	0.5	0.5	100
	6.25		200	0.5	0.5	1.0	110
	12.5		200	0	0	0.5	116
	25		200	0	0	1.0	101
	50		200	0	0	1.5	114
	100		200	0	0	25.5	104
MMC	0.12		200	59.5	59.5	0	
MWNT-7	0	+ (Short-term) ^a	200	0.5	0	0.5	100
	6.25		200	1.0	0.5	0.5	109
	12.5		200	0.5	0	1.0	111
	25		200	1.5	0.5	0	98
	50		200	1.0	0.5	0.5	104
	100		200	0.5	0.5	2.5	113
B[a]P	30		200	41.0	40.0	0	
MWNT-7	0	– (Continuous) ^b	200	0	0	1.0	100
	6.25		200	0.5	0.5	0	101
	12.5		200	0.5	0	0.5	103
	25		200	0	0	1.0	101
	50		200	0	0	1.5	106
	100		200	0.5	0	20.5	97
MMC	0.06		200	54.0	51.0	1.0	

MMC: Mitomycin C.

B[a]P: Benzo[a]pyrene.

^a Exposure time was 6 h, and recovery time was 18 h.^b Exposure time was 24 h.

Applicant's summary and conclusion

Interpretation of results

positive

Conclusions

Slightly increase and strong increase of numerical chromosome aberration was observed at 100 µg/mL of N-MWCNTs and MWNT-7 without S9 mix, respectively.

Endpoint study record: Mitsui MWNT-7: Genetic toxicity in vitro.003**Administrative Data**

Purpose flag supporting study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to OECD TG 476

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Asakura M, Sasaki T, Sugiyama T, Takaya M, Koda S, Nagano K, Arito H, Fukushima S.	2010	Genotoxicity and Cytotoxicity of Multi-wall Carbon Nanotubes in Cultured Chinese Hamster Lung Cells in Comparison with Chrysotile A Fiber	J. Occup Health, 52: 155-166, (2010)	Japan Bioassay Reserch Center				

Materials and methods**Type of genotoxicity**

gene mutation

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWCNT

Test material form

no data

Details on test materialMean and SD of the length: $5.0 \pm 4.5 \mu\text{m}$ Mean and SD of the width: $88 \pm 5 \text{ nm}$ **Method****Target gene**

hgprr locus

Species/strain**Species/strain** other: Chinese hamster lung CHL/IU cells**Metabolic** without
activation**Test concentrations**6.3, 12.5, 25, 50, 100 $\mu\text{g/mL}$ of the test substance**Vehicle**

DMSO or ultra-pure water

Controls**Solvent / vehicle controls** yes (DMSO or ultra-pure water)**Positive controls** yes**Positive control substance** other: ethyl methanesulfonate (EMS)**Any other information on materials and methods incl. tables**

Three-hundred thousand CHL/IU cells (30,000 cells/ml x 10 ml) were seeded in a 100-mm plastic culture dish, incubated in a culture medium for 24 h, and then replaced with the test substance suspended in culture medium. The cells were exposed to MWCNT at 6.3 to 100 $\mu\text{g/ml}$ or chrysotile at 1.56 to 25 $\mu\text{g/ml}$ for 48 h. DMSO at 0.5% or ultra-pure water at 5% served as a negative control and at 200 $\mu\text{g/ml}$ as a positive control. Treatment concentrations of MWCNT or chrysotile were determined on the basis of the preliminary mutation assay. Then, the cells were rinsed with PBS and incubated in normal medium for a mutation expression time of 6 days. After the 6-day incubation, the cells were treated with trypsin, and 40,000 cells were transferred to each of twenty 60-mm culture dishes in medium containing 6-thioguanine (6-TG) for mutation selection. One hundred cells transferred to each of three 60-mm culture dishes in normal medium were used for cell viability assessment. After incubating for 10 days, the cell colonies formed were fixed with ethanol and stained with 5% Giemsa, and the number of 6-TG-resistant colonies was counted. The mutation frequency rate was expressed as the scored number of 6-TG-resistant cells per 10^6 cells corrected by the cell viability.

Results and discussions

Test results

Species/strain other: Chinese hamster lung CHL/IU cells

Metabolic activation without

Genotoxicity negative

Cytotoxicity yes

Vehicle controls valid yes

Positive controls valid yes

Any other information on results incl. tables

The MWCNT was negative for mutagenicity at *hgp* locus gene mutation in CHL/IU cells, although the cell viabilities decreased in a dose depending manner.

Overall remarks, attachments

Attached background material

Illustration (picture/graph)

Table 3. Mutagenicity of MWCNT and chrysotile at the *hgp* locus with CHL/IU cells

Concentration ($\mu\text{g/ml}$)	Viability(%)	Mutation rate per 10^6 cells
MWCNT		
0	100	4.68
6.3	86	3.56
12.5	63	6.83
25	48	4.77
50	33	6.14
100	21	3.82
EMS	33	462*
Chrysotile		
0	100	5.53
1.56	73	7.38
3.13	54	6.63
6.25	43	5.33
12.5	23	2.32
25	17	1.53
EMS	48	325*

EMS: ethyl methanesulfonate at 200 $\mu\text{g/ml}$. *: Positive response.

Applicant's summary and conclusion**Interpretation of results**

negative

Conclusions

The test substance MWCNT did not induce hprt gene mutation to CHL/IU cells without metabolic activation.

Endpoint study record: Mitsui MWNT-7: Genetic toxicity in vitro.004**Administrative Data**

Purpose flag supporting study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to OECD TG 473

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Asakura et al.	2010	Genotoxicity and Cytotoxicity of Multi-wall carbon Nanotubes in Cultured Chinese Hamster Lung Cells in Comparison with Chrysotile A Fibers	J. Occup Health, 52, 155-166, (2010)	Japan Bioassay Research Center				

Materials and methods**Type of genotoxicity**

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance

no data

Test materials

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Details on test material

Mean and SD of the length: $5.0 \pm 4.5 \mu\text{m}$ Mean and SD of the width: $88 \pm 5 \text{ nm}$

Method

Species/strain

Species/strain other: Chinese hamster lung CHL/IU cells

Metabolic activation without

Test concentrations

24 hrs continuous treatment without metabolic activation: 1.3, 5.0, 20, 80 $\mu\text{g/ml}$; 48 hrs continuous treatment without metabolic activation: 0.078, 0.31, 1.3, 5.0 $\mu\text{g/ml}$

Vehicle

DMSO or ultra-pure water

Controls

Solvent / vehicle controls yes (DMSO or ultra-pure water)

Positive controls yes

Positive control substance mitomycin C

Any other information on materials and methods incl. tables

One hundred thousand CHL/IU cells ($20,000 \text{ cells/ml}$ - 5 ml) were seeded in a 60-mm plastic culture dish, incubated in a culture medium for 24 h, and then replaced with test substance suspended in culture medium. The cells were exposed to MWCNT at 0.078 to 80 $\mu\text{g/ml}$ or chrysotile at 0.8 to 20 $\mu\text{g/ml}$ for 24 or 48 h. DMSO at 0.5% or ultra-pure water at 5% served as a negative control and MMC at 0.04 $\mu\text{g/ml}$ as a positive control. Treatment concentrations of MWCNT or chrysotile were determined on the basis of the preliminary chromosome aberration assay. For chromosome preparation, colcemid at a final concentration of 0.2 $\mu\text{g/ml}$ was added to the culture medium 2 h before cell harvesting. Chromosomes were prepared by the air-drying method and stained with 2% Giemsa. The cytotoxicity was assessed by counting the trypan blue-stained cells as the dead ones, and expressed as a growth index by dividing the number of viable cells after 24- or 48-hour treatment with MWCNT or chrysotile by that of the respective negative control. The frequency of the cells with various types of structural aberrations including chromatid break, chromatid exchange, chromosome break, chromosome exchange and others (fragmentations except pulverization), for each dose in a 200 well-spread metaphase (100 metaphase/culture), as well as the cells with numerical aberration (polyploidy) were scored.

Results and discussions

Test results

Species/strain other: Chinese hamster lung CHL/IU cells

Metabolic activation without

Genotoxicity positive (polyploids)

Vehicle controls valid yes

Positive controls valid yes

Any other information on results incl. tables

The MWCNT did not induce structural chromosome aberration. On the other hand, a significantly increased number of cells showing polyploidy was observed for the MWCNT at 5 µg/ml and above in the 24-hours treatment and at 1.3 and 5 µg/ml in the 48-hours treatment.

Overall remarks, attachments

Attached full study report

Illustration (picture/graph)

Table 1. Structural and numerical chromosome aberrations of MWCNT and chrysotile with CHL/IU cells

Treatment time (h)	Concentration (µg/ml)	Percent (%) of cells showing structural chromosome aberrations							Percent (%) of cells showing polyploids		Growth index (%)
		Cell No.	ctb	cte	csb	cse	other	total	Cell No.	polyploidy	
MWCNT											
24	0	200	0	0	0	0	0	0	201	0.5	100
	1.3	200	0	0	0	0	0	0	203	1.5	76
	5.0	200	0	0	0	0	0	0	207	3.4*	62
	20	200	0	0	0	0	0	0	234	14.5**	56
	80	200	0	0	0	0	0	0	242	17.4**	50
	MMC	200	7	23.5	0	0	0	ns ^{a)} 28.5**	200	p<0.01 ^{a)} 0	—
48	0	200	0.5	0	0	0	0	0.5	202	1.0	100
	0.078	200	0.5	0	0	0	0	0.5	203	1.5	83
	0.31	200	0.5	0	0	0	0	0.5	204	2.0	74
	1.3	200	0	0	0	0	0	0	217	7.8**	67
	5.0	200	0	0	0	0	0	0	261	23.4**	59
	MMC	200	6.5	58.5	0	0	0	ns ^{a)} 63**	200	p<0.01 ^{a)} 0	—
Chrysotile											
24	0	200	0	1.5	0.5	0	0	2.0	201	0.5	100
	0.8	200	0.5	0.5	0	0	0	1.0	209	4.3*	80
	4.0	200	0.5	1.5	0	0	0	2.0	238	22.5**	69
	20	200	0	0	0	0	0	0	304	34.2**	42
	MMC	200	11	30	0	0	0	ns ^{a)} 38.5**	201	p<0.01 ^{a)} 0.5	—
48	0	200	0.5	0	0	0	0	0.5	201	0.5	100
	0.8	200	0.5	0.5	0	0	0	1.0	208	3.8*	96
	4.0	200	1.0	1.0	0	0.5	0	2.5	224	10.7**	81
	20	200	1.0	0.5	0	0.5	0	2.0	330	39.4**	42
	MMC	200	13	35.5	0	0.5	0	ns ^{a)} 41.5**	201	p<0.01 ^{a)} 0.5	—

Fisher's exact test: * $p < 0.05$, ** $p < 0.01$. a) Cochran-Armitage test. ctb: chromatid break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; others: fragmentation etc. (except pulverization); MMC: Mitomycin C at 0.04 µg/ml.

Applicant's summary and conclusion**Conclusions**

The test substance MWNT-7 induced polyploids to CHL/IU cells with statistical significance in 24 hrs treatment and 48 hrs treatment without metabolic activation.

Endpoint study record: Mitsui MWNT-7: Genetic toxicity in vitro.005**Administrative Data**

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability incl. deficiencies Acceptable, well-documented publication

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Asakura M, Sasaki T, Sugiyama T, Takaya M, Koda S, Nagano K, Arito H, Fukushima S.	2010	Genotoxicity and Cytotoxicity of Multi-wall Carbon Nanotubes in Cultured Chinese Hamster Lung Cells in Comparison with Chrysotile A Fiber	J. Occup Health, 52: 155-166, (2010)	Japan Bioassay Research Center				

Materials and methods**Type of genotoxicity**

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWCNT

Test material form

no data

Method**Species/strain**

Species/strain other: Chinese hamster lung CHL/IU cells

Metabolic activation without

Test concentrations

0.02, 0.078, 0.31, 1.3, 5.0 µg/ml

Vehicle

DMSO or ultra-pure water

Controls

Solvent / vehicle controls yes (DMSO or ultra-pure water)

Positive controls yes

Positive control substance mitomycin C

Any other information on materials and methods incl. tables

Twenty-four thousand CHL/IU cells (12,000 cells/ml x 2 ml) were seeded in a 35-mm plastic culture dish, incubated in a culture medium for 24 h, and then replaced with the test substance suspended in culture medium. The cells were exposed to MWCNT at 0.02 to 5.0 µg/ml or chrysotile at 0.1 to 1.6 µg/ml for 48 h. DMSO at 0.5% or ultra-pure water at 5% served as a negative control and MMC at 0.01 µg/ml as a positive control. Treatment concentrations of MWCNT or chrysotile were determined on the basis of the preliminary micronucleus assay. The cells were washed with PBS, fixed with 10% formaline and stained by mounting with 40µg/ml acridine orange and 10 µg/ml DAPI solution. Immediately after the staining, the cells were observed with fluorescence microscopy using blue excitation, and evaluated for the number of cells having micronuclei, bi-nuclei and multi-nuclei having more than two nuclei in 2,000 intact interphase cells (1,000 cells/culture). The cytotoxicity was expressed as a growth index by dividing the number of viable cells after the 48-hour treatment with MWCNT or chrysotile by that of the respective negative control. Micronucleus was defined as having less than one-fourth of the diameter of the main nucleus. Numbers of bi-nucleated cells and mitotic cells as well as multi-nucleated cells having more than two nuclei appearing in the same microscopic field were also counted.

Results and discussions

Test results

Species/strain	other: Chinese hamster lung CHL/IU cells
Metabolic activation	without
Genotoxicity	positive
Vehicle controls valid	yes
Positive controls valid	yes

Any other information on results incl. tables

No statistically significant increase in the number of micronucleated cells was found at any dose level of the MWCNT, although statistically significant dose-dependent micronucleus induction exhibited as indicated by the Cochran-Armitage test. The MWCNT significantly increased the number of bi-nucleated cells at 0.31 µg/ml and above, and multi nucleated cells at 1.3 µg/ml and above.

Overall remarks, attachments

Attached full study report

Illustration (picture/graph)

Table 2. Micronucleus assay of MWCNT and chrysotile with CHL/IU cells

Concentration ($\mu\text{g}/\text{ml}$)	MN (%)	Other relevance (%)			Growth Index (%)
		Bi-N	Multi-N	MP	
MWCNT					
0	8.5	4.5	2.5	14.5	100
0.02	10.5	3.0	2.5	12.5	94
0.078	9.5	6.0	2.5	11.5	88
0.31	12.0	19.0**	5.0	12.0	79
1.3	13.0	27.5**	10.5**	10.0	73
5.0	14.0	63.5**	21.5**	9.5	63
Cochran- Armitage test	$p<0.05$	$p<0.01$	$p<0.01$	–	–
MMC	48.5**	9.5	5.0	9.5	–
Chrysotile					
0	16.0	4.0	2.5	30.0	100
0.1	17.0	4.0	4.5	27.5	98
0.2	22.5	7.5	4.5	25.5	99
0.4	24.0*	40.0**	12.5**	20.5	89
0.8	24.5*	52.5**	14.0**	22.0	86
1.6	30.5**	68.0**	32.5**	23.0	84
Cochran- Armitage test	$p<0.01$	$p<0.01$	$p<0.01$	–	–
MMC	82.0**	5.5	8.5**	21.5	–

Fisher's exact test. * $p<0.05$, ** $p<0.01$. MN: micronucleated cell; Bi-N: bi-nucleated cell; Multi-N: multi-nucleated cell having more than two nuclei; MP: mitotic cell; MMC: Mitomycin C at 0.01 $\mu\text{g}/\text{ml}$. Observed number of cells was 2,000 cells per dose.

Applicant's summary and conclusion

Conclusions

MWCNT exerted marginal but significant induction of micronuclei to CHL/IU cells without metabolic activation.

Endpoint study record: Nanocyl NC7000: Genetic toxicity in vitro.001**Administrative Data**

Purpose flag disregarded study
Study result type no data
Reliability 4 (not assignable)
Rationale for reliability incl. deficiencies Summary data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Nanocyl	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Materials and methods**Type of genotoxicity**

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Nanocyl NC 7000

Applicant's summary and conclusion**Interpretation of results**

negative

Conclusions

Not mutagenic up to the highest possible dose i.e. 2000µg/plate

Endpoint study record: Nikkiso: Genetic toxicity in vitro.001**Administrative Data**

Purpose flag key study
Study result type experimental result **Study period** 2010
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to OECD TG471

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J.	2012	Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of in vitro and in vivo assays.	Regul Toxicol Pharmacol., 63(2):188-195, (2012)					
study report	AIST	2010	Bacterial reverse mutation assay of Multi-walled Carbon Nanotubes B		Ina Research	ZT10125			

Data access

data published

Materials and methods**Type of genotoxicity**

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other:	Nikkiso N-MWCNTs

Test material form

powder

Details on test material

- Name of test material (as cited in study report): N-MWCNTs (Manufactured by Nikkiso Co.,Ltd. (Japan))
- Analytical purity: No data
- Impurities (identity and concentrations): Gallium: 176 ppm, Aluminum: 80 ppm, Iron: 53 ppm, Cadmium: 16 ppm, Lithium: 0.5 ppm
- Other:
BET surface area: 69 m²/g, Mean diameter: 44 nm

Method**Species/strain****Species/strain** other: S. typhimurium TA100, TA98, TA1535, TA1537; E. coli WP2uvrA**Metabolic activation** with and without**Metabolic activation system** S9 mix**Test concentrations**

1.56, 3.13, 6.25, 12.5, 25, 50, 100 ug/plate

Vehicle

- Vehicle(s)/solvent(s) used: 0.3%CMC-Na (sodium carboxymethyl cellulose)

Controls**Positive controls** yes**Positive control substance** sodium azide**Positive controls** yes**Positive control substance** other: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide**Positive controls** yes**Positive control substance** other: 9-aminoacridine
hydrochloride**Positive controls** yes**Positive control substance** other: 2-aminoanthracene**Solvent / vehicle controls** yes (0.3% CMC)**Details on test system and conditions**

Pre-incubation method with and without metabolic activation (S9 mix) was used.

Evaluation criteria

According to Ames et al., 1975 and US-FDA, 2000.

Statistics

No statistical analysis.

Results and discussions**Test results**

Species/strain other: *S. typhimurium* TA100, TA98, TA1535, TA1537 and *E. coli* WP2uvrA

Metabolic activation with and without

Genotoxicity negative

Vehicle controls valid yes

Positive controls valid yes

Any other information on results incl. tables

No strains showed the growth inhibition with or without metabolic activation. No mutation induction was found with or without metabolic activation in all concentrations and in all strains.

Table. Result of bacterial reverse mutation tests on N-MWCNT

Test substance	concentration (µg/plate)	S9 mix	No. of revertant colonies/plate (Mean)					
			Base substitution type			Frameshift type		
			Ta100	TA1535	WP2uvrA	TA98	TA1537	
N-MWCNTs	0	-	120	15	26	13	13	
	1.56		109	19	27	12	10	
	3.13		119	20	24	16	11	
	6.25		116	19	24	14	13	
	12.5		114	17	25	16	11	
	25		109	16	19	15	10	
	50a)		114	23	19	14	12	
	100a)		117	20	21	14	9	
AF-2	0.01	+	399		108			
	0.1					535		
NaN3	0.5			413				
9AA	80						271	
N-MWCNTs	0		105	25	18	23	19	
	1.56		115	21	29	31	18	
	3.13		114	19	24	24	12	
	6.25		127	20	20	27	15	
	12.5		133	20	19	26	21	
	25		120	20	23	22	17	
	50a)		125	24	27	25	16	
	100a)		128	17	19	22	17	
2AA	0.5					461		
	1		713					
	2			277			203	
	10				563			

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide

NaN3: Sodium azide

9AA: 9-aminoacridine hydrochloride

2AA: 2-Aminoanthracene

a: Precipitation

Overall remarks, attachments

Attached full study report

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

It was judged that CNT-B possesses no potency to induce gene mutation in the tested 5 bacterial strains under the present test conditions.

Executive summary

The test substance, CNT-B did not increase the numbers of bacterial revertant colonies twice or more those of the solvent control in any of the the bacterial strains, and a reproducibility of the results was found in the preliminary test and two main tests with or without metabolic activation.

Endpoint study record: Nikkiso: Genetic toxicity in vitro.002

Administrative Data

Purpose flag	key study
Study result type	experimental result Study period 2010-2011
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to OECD TG 473

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J.	2012	Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of in vitro and in vivo assays.	Regul Toxicol Pharmacol., 63(2):188-195, (2012)					
study report	AIST	2011	Chromosomal aberration test of Multiwalled Carbon Nanotubes B (CNT-B)		Ina Research	ZT10126			

Materials and methods**Type of genotoxicity**

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Nikkiso N-MWCNTs

Test material form

powder

Details on test material

- Name of test material (as cited in study report): N-MWCNTs (Manufactured by Nikkiso Co.,Ltd. (Japan))
- Analytical purity: No data
- Impurities (identity and concentrations): Gallium: 176 ppm, Aluminum: 80 ppm, Iron: 53 ppm, Cadmium: 16 ppm, Lithium: 0.5 ppm

- Other:
BET surface area: 69 m²/g, Mean diameter: 44 nm

Method

Species/strain

Species/strain other: Chinese hamster lung fibroblasts (CHL/IU cells)

Metabolic activation with and without

Metabolic activation system S9 mix

Test concentrations

12.5, 25, 50, 100 µg/ml

Vehicle

- Vehicle(s)/solvent(s) used: 0.3%CMC-Na (sodium carboxymethyl cellulose)

Controls

Solvent / vehicle controls yes (0.3%CMC-Na)

Positive controls yes

Positive control substance mitomycin C

Positive controls yes

Positive control substance benzo(a)pyrene

Details on test system and conditions

Treatment: Short-term treatment methods of 6hrs with and without metabolic activation and a continuous treatment method without metabolic activation were conducted.

Evaluation criteria

The clastogenic potential was judged as negative, equivocal, and positive if the incidence of cells showing any aberration was less than 5%, 5-10%, more than 10%, respectively.

Results and discussions

Test results

Species/strain other: CHL/IU cells

Metabolic activation with and without

Genotoxicity positive

Cytotoxicity no

Vehicle controls valid yes

Positive controls valid yes

Any other information on results incl. tables

Slightly increase of numerical chromosome aberration was observed at 100 µg/mL of N-MWCNTs without S9 mix, respectively. No increase of structural chromosome aberration was observed in any groups tested with and without metabolic activation.

Table. Results of chromosomal aberration test on MWNT-7 in a Chinese hamster lung cell line

S9 mix (Duration of exposure)	Dose	No. of cells	% Structural aberrations		% Polyploid cells	Growth rate (%)
	(µg/mL)		+gap	-gap		
- (Short term)a	0	200	0	0	0.5	100
	12.5	200	0	0	0.5	108
	25	200	0	0	1	107
	50	200	1.5	1	1	98
	100	200	0.5	0.5	15	103
	MMC 0.12	200	58	58	0	-
-+(Short term)a	0	200	1.5	0	0.5	100
	12.5	200	1	0	0	94
	25	200	0	0	0	98
	50	200	1	0.5	1.5	89
	100	200	3	2.5	2	79
	B[a]P 30	200	46.5	45	0.5	-
- (Continuous)b	0	200	1	1	0.5	100
	12.5	200	0.5	0.5	0.5	112
	25	200	1	1	1	104
	50	200	1.5	0.5	2.5	97
	100	200	1	0.5	6.5	96
	MMC 0.06	200	54	53	0	-

MMC: Mitomycin C

B[a]P: Benzo[a]pyrene

a Exposure time 6h, and recovery time was 18h

b Exposure time was 24h

Overall remarks, attachments**Attached full study report****Applicant's summary and conclusion****Interpretation of results**

positive without metabolic activation

Conclusions

It was judged that the test substance N-MWCNTs slightly induced chromosome aberration of polyploidy to CHL/IU cells in the treatment without metabolic activation under the present test conditions.

Endpoint study record: Hanwha CM-100: Genetic toxicity in vitro.001**Administrative Data**

Purpose flag supporting study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to OECD TG 471

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
secondary source	National institute of environmental research, Ministry of Environment, Korea	2009	The production of multi-walled carbon nanotubes(MWCNTs) hazardous data						
publication	Kim JS, Lee K, Lee YH, Cho HS, Kim KH, Choi KH, Lee SH, Song KS, Kang CS, Yu IJ.	2011	Aspect ratio has no effect on genotoxicity of multi-wall carbon nanotubes	Arch Toxicol., 85: 775-786, (2011)					

Materials and methods**Type of genotoxicity**

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	

GLP compliance

yes

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Hanwha CM-95

Details on test material

- Name of test material (as cited in study report): CM-95 (Manufactured by Hanwha Nanotech Inc. (Korea))
 - Analytical purity: 95%
 - Impurities (identity and concentrations): Iron: approx. 5%
 - Other
- Diameter: 10-15 nm, Length: ~20 µm

Method**Species/strain**

Species/strain other: Salmonella typhimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP2uvrA

Metabolic activation with and without

Metabolic activation system S9 mix

Test concentrations

Tests with S9 mix: 0, 5, 10, 21, 42, 83, 167 and 333 µg/plate for Salmonella typhimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP2uvrA;

Tests without S9 mix: 0, 5, 10, 21, 42, 83, 167 and 333 µg/plate for Salmonella typhimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP2uvrA

Vehicle

DMSO

Controls

Solvent / vehicle controls yes (DMSO)

Positive controls yes

Positive control substance other: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) (CAS No. 3688-53-7)

Solvent / vehicle controls yes (DMSO)

Positive controls yes

Positive control substance sodium azide

Solvent / vehicle controls yes (DMSO)

Positive controls yes

Positive control substance other: 2-aminoanthracene (2-AA; CAS No.613-13-8)

Details on test system and conditions

Positive control groups and treatment: Positive control groups were treated as followings; In the absence of S9 mix, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2; CAS No. 3688-53-7) for TA100 and WP2uvrA, Sodium azide (CAS No. 26628-22-8) for TA1535. In the presence of S9 mix, 2-aminoanthracene (2-AA; CAS No.613-13-8) for all tested bacterial species.

NUMBER OF REPLICATIONS:3

All the chemicals were dissolved in dimethylsulfoxide and treated to bacteria along with the test substance.

Any other information on materials and methods incl. tables**Test Design:**

Number of replicates: 3

Frequency of Dosing: Single treatment

Positive control groups and treatment: Positive control groups were treated as followings; In the absence of S9 mix, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2; CAS No. 3688-53-7) for TA100 and WP2uvrA, Sodium azide (CAS No. 26628-22-8) for TA1535. In the presence of S9 mix, 2-aminoanthracene (2-AA; CAS No.613-13-8) for all tested bacterial species. All the chemicals were dissolved in dimethylsulfoxide and treated to bacteria along with the test substance.

Number of metaphases analyzed: Not applicable.

Results and discussions**Test results**

Species/strain other: Salmonella typhimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP2uvrA

Metabolic activation with and without

Genotoxicity negative

Cytotoxicity no

Vehicle controls yes
valid

Positive controls yes
valid

Any other information on results incl. tables

Table 5.1. The number of base substitutional revertant colonies for MWCNTs in the absence of S9 mix (main test).

Concentration($\mu\text{g}/\text{plate}$)	Types of Bacteria (Mean $\pm$ S.D.)		
	TA100	TA1535	WP2uvrA
0	173 $\pm$ 3.5	7 $\pm$ 2.5	39 $\pm$ 1.7
5	148 $\pm$ 9.7	7 $\pm$ 4.0	31 $\pm$ 2.5
10	160 $\pm$ 14.0	6 $\pm$ 1.2	36 $\pm$ 2.5
21	151 $\pm$ 5.5	5 $\pm$ 2.0	37 $\pm$ 7.8
42	151 $\pm$ 6.9	9 $\pm$ 0.6	34 $\pm$ 6.1
83	149 $\pm$ 15.7	8 $\pm$ 1.0	37 $\pm$ 2.1
167	142 $\pm$ 11.5	9 $\pm$ 1.2	32 $\pm$ 10.4
333†	146 $\pm$ 12.5	8 $\pm$ 1.5	30 $\pm$ 9.9
Positive control	500 $\pm$ 17.0 ¹⁾	283 $\pm$ 18.0 ²⁾	255 $\pm$ 32.1 ³⁾

†: aggregation and precipitation of test substance

1) : 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide(AF-2) 0.01 $\mu\text{g}/\text{plate}$

2) : Sodium azide(NaN_3) 0.5 $\mu\text{g}/\text{plate}$

3) : 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide(AF-2) 0.01 $\mu\text{g}/\text{plate}$

Table 5.2. The number of frameshift revertant colonies for MWCNTs in the absence of S9 mix (main test).

Concentration($\mu\text{g}/\text{plate}$)	Types of Bacteria (Mean $\pm$ S.D.)	
	TA98	TA1537
0	9 $\pm$ 2.1	6 $\pm$ 0.6
5	10 $\pm$ 3.1	5 $\pm$ 2.1
10	13 $\pm$ 4.0	5 $\pm$ 1.7
21	11 $\pm$ 1.2	6 $\pm$ 2.5
42	8 $\pm$ 3.2	6 $\pm$ 0.6
83	12 $\pm$ 1.5	3 $\pm$ 1.2
167	9 $\pm$ 2.5	3 $\pm$ 1.5
333†	11 $\pm$ 3.0	4 $\pm$ 2.3
Positive control	557 $\pm$ 40.1 ¹⁾	2434 $\pm$ 128.6 ²⁾

†: aggregation and precipitation of test substance

1) : 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide(AF-2)

0.1 $\mu\text{g}/\text{plate}$

2) :9-Aminoacridine hydrochloride hydrate (9-AA) 80 $\mu\text{g}/\text{plate}$

Table 5.3. The number of base substitutional revertant colonies for MWCNTs in the present of S9 mix (main test).

Concentration($\mu\text{g}/\text{plate}$)	Types of Bacteria (Mean $\pm$ S.D.)		
	TA100	TA1535	WP2uvrA
0	154 $\pm$ 13.5	8 $\pm$ 3.6	41 $\pm$ 4.9
5	161 $\pm$ 7.2	9 $\pm$ 2.3	39 $\pm$ 6.9
10	158 $\pm$ 8.3	9 $\pm$ 2.1	46 $\pm$ 6.2
21	138 $\pm$ 11.0	8 $\pm$ 1.7	46 $\pm$ 9.3
42	144 $\pm$ 22.4	7 $\pm$ 4.0	46 $\pm$ 6.2
83	185 $\pm$ 4.2	14 $\pm$ 4.5	52 $\pm$ 3.6
167	168 $\pm$ 7.2	7 $\pm$ 2.9	41 $\pm$ 8.6
333†	142 $\pm$ 21.7	11 $\pm$ 3.6	40 $\pm$ 6.4
Positive control	1994 $\pm$ 43.4 ¹⁾	253 $\pm$ 16.2 ²⁾	352 $\pm$ 49.3 ³⁾

†: aggregation and precipitation of test substance

1) :2-Aminoanthracene(2AA) 1.0 $\mu\text{g}/\text{plate}$

2) :2-Aminoanthracene(2AA) 2.0 $\mu\text{g}/\text{plate}$

3)2-Aminoanthracene(2AA) 10.0 $\mu\text{g}/\text{plate}$

Table 5.4. The number of frameshift revertant colonies for MWCNTs in the presence of S9 mix (main test).

Concentration (µg/plate)	Types of Bacteria (Mean ± S.D.)	
	TA98	TA1537
0	14 ± 4.6	9 ± 3.6
5	15 ± 2.5	6 ± 1.7
10	15 ± 4.4	6 ± 2.0
21	11 ± 1.0	7 ± 3.0
42	12 ± 4.0	8 ± 4.0
83	18 ± 5.0	5 ± 2.0
167	12 ± 4.5	3 ± 2.3
333†	15 ± 3.0	6 ± 2.1
Positive control	447 ± 22.0 ¹⁾	257 ± 29.0 ²⁾

†: aggregation and precipitation of test substance

1) : 2-Aminoanthracene(2AA) 1.0µg/plate

2) : 2-Aminoanthracene(2AA) 2.0µg/plate

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

MWCNTs did not induce an increase in bacterial colony formation, and thus were not mutagenic under the test conditions.

Endpoint study record: Hanwha CM-100: Genetic toxicity in vitro.002

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to OECD TG 473

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
secondary source	National institute of environmental research, Ministry of Environment, Korea	2009	The production of multi-walled carbon nanotubes(MWCNTs) hazardous data						
publication	Kim JS, Lee K, Lee YH, Cho HS, Kim KH, Choi KH, Lee SH, Song KS, Kang CS, Yu IJ.	2011	Aspect ratio has no effect on genotoxicity of multi-wall carbon nanotubes	Arch Toxicol., 85: 775-786, (2011)					

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other:	Hanwha CM-95

Details on test material

- Name of test material (as cited in study report): CM-95 (Manufactured by Hanwha Nanotech Inc. (Korea))
- Analytical purity: 95%
- Impurities (identity and concentrations): Iron: approx. 5%
- OtherDiameter: 10-15 nm, Length: ~20 µm

Method

Species/strain

Species/strain

Chinese hamster Ovary (CHO)

Details on mammalian cell lines (if applicable)

Chinese hamster ovary fibroblast, CHO-K1

Metabolic activation

with and without

Test concentrations

Concentrations tested: Tests without S9 mix: 1.563, 3.125, 6.25 µg/mL for 24 hours treatment and 6 hours recovery period. Tests with S9 mix: 6.25, 12.5 25 µg/mL

Vehicle

1.0% citrate solution

Controls

Negative controls	yes (untreated control)
Solvent / vehicle controls	yes (1.0% citrate solution)
Positive controls	yes
Positive control substance	mitomycin C cyclophosphamide

Details on test system and conditions

Test conditions: Number of replicates: Two slides were prepared from each plate.

Frequency of Dosing: single treatment

Positive and negative control groups and treatment: For positive control, 0.04µg/mL of mitomycin C (in the absence of S9 mix) and 10µg/mL of cyclophosphamide-H₂O (in the presence of S9 mix) were treated to cells. For negative control, cells were treated with 1.0% citrate solution.

Number of metaphases analyzed: Not applicable Solvent: Sterilized distilled water

Description of follow up repeat study: Follow up study was performed because metaphasic cells were not observed in the first test. with and without metabolic activation

Evaluation criteria

Criteria for evaluating

Results: Statistical analysis was performed to determine the increase in frequency of aberrant metaphase and polyploidy. A concentration-related increase or a reproducible increase in the number of cells with chromosome aberrations was considered to be positive.

Statistics

Fisher's exact test

Results and discussions**Test results**

Species/strain Chinese hamster Ovary (CHO)

Metabolic activation with and without

Genotoxicity negative

Cytotoxicity no

Vehicle controls valid yes

Negative controls valid yes

Positive controls valid yes

Any other information on results incl. tables

Table 5.3. The number of cells with chromosome aberrations in the absence of S9 mix for MWCNTs (24 hrs exposure).

Dose (µg/mL)	No. of cells	Types of Aberration						No. of total chromosome aberrations		No. of cells with chromosome aberrations	
		ctb	cte	ctb	cte	PP	Gap	(-)Gap	(+)Gap	(-)Gap	(+)Gap
U.C.	100	0	0	0	0	0	1	0	1	0	1
	100	0	0	0	0	0	0	0	0	0	0
N.C.	100	1	0	0	0	0	0	1	1	1	1
	100	0	0	0	0	0	1	0	1	0	1
1.563	100	0	0	0	0	0	0	0	0	0	0
	100	1	0	0	0	0	0	1	1	1	1
3.125	100	0	1	0	0	0	0	1	1	1	1
	100	0	0	0	0	0	0	0	0	0	0
6.25	100	2	0	0	0	0	1	2	3	2	3
	100	1	0	0	0	0	0	1	1	1	1
MMC	100	7	22	1	0	0	0	30	30	29 *	29
	100	4	26	0	0	0	1	30	31	29 *	30

* : significantly different from the negative control at $p < 0.05$ U.C. : Untreated control N.C. : Negative control

MMC : Mitomycin C (0.04 µg/mL) ctb : Chromatid type breakage cte : Chromatid type exchange csb : Chromosome type breakage

cse : Chromosome type exchange PP : Polyploidy ER : Endoreduplication

Table 5.4. The number of cells with chromosome aberrations in the absence of S9 mix for MWCNTs (6 hrs exposure).

Dose (µg/mL)	No. of cells	Types of Aberration						No. of total chromosome aberrations		No. of cells with chromosome aberrations	
		ctb	cte	ctb	cte	PP	Gap	(-)Gap	(+)Gap	(-)Gap	(+)Gap
U.C.	100	0	0	0	0	0	1	0	1	0	1
	100	0	0	0	0	0	0	0	0	0	0
N.C.	100	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	1	0	1	0	1
1.563	100	1	0	0	0	0	1	1	2	1	2
	100	1	0	0	0	0	1	1	2	1	2
3.125	100	1	0	0	0	0	1	1	2	1	2
	100	0	0	0	0	0	0	0	0	0	0
6.25	100	0	0	0	0	0	1	0	1	0	1
	100	1	0	0	0	0	0	1	1	1	1
MMC	100	5	19	0	0	0	1	24	25	24 *	25
	100	5	22	1	0	0	2	28	30	27 *	29

* : significantly different from the negative control at $p < 0.05$ U.C. : Untreated control N.C. : Negative control

MMC : Mitomycin C (0.04 µg/mL) ctb : Chromatid type breakage ste : Chromatid type exchange csb : Chromosome type breakage cse : Chromosome type exchange PP : Polyploidy ER : Endoreduplication

Table 5.5. The number of cells with chromosome aberrations in the presence of S9 mix for MWCNTs (6 hrs exposure).

Dose (µg/mL)	No. of cells	Types of Aberration						No. of total chromosome aberrations		No. of cells with chromosome aberrations	
		ctb	cte	ctb	cte	PP	Gap	(-)Gap	(+)Gap	(-)Gap	(+)Gap
U.C.	100	0	0	0	0	0	0	0	0	0	0
	100	1	0	0	0	0	1	1	2	1	2
N.C.	100	0	0	0	0	0	0	0	0	0	0
	100	1	0	0	0	0	0	1	1	1	1
6.25	100	0	1	0	0	0	1	1	2	1	2
	100	1	0	0	0	0	0	1	1	1	1
12.5	100	1	1	0	0	0	0	2	2	2	2
	100	0	0	0	0	0	1	0	1	0	1
25	100	1	0	0	0	0	0	1	1	1	1
	100	0	0	0	0	0	0	0	0	0	0
CPP	100	5	27	0	0	0	2	32	34	32 *	34
	100	2	32	0	0	0	1	34	35	31 *	32

* : significantly different from the negative control at $p < 0.05$ U.C. : Untreated control N.C. : Negative control

CPA : Cyclophosphamide • H₂O (10 µg/mL) ctb : Chromatid type breakage ste : Chromatid type exchange

csb : Chromosome type breakage cse : Chromosome type exchange PP : Polyploidy ER : Endoreduplication

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

MWCNTs did not induce an increase in chromosomal aberration. Therefore, it was not genotoxic under the test conditions.

7.6.2 Genetic toxicity in vivo

Endpoint study record: Mitsui MWNT-7: Genetic toxicity in vivo.001

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies According to OECD TG 474

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J.	2012	Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of in vitro and in vivo assays	Regul Toxicol Pharmacol., 63(2):188-195, (2012)	Ina Research Inc.				

Materials and methods**Type of genotoxicity**

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Test material form

no data

Details on test material

- Name of test material (as cited in study report): MWNT-7 (Manufactured by Mitsui & Co.,Ltd. (Japan))
- Analytical purity: No data
- Impurities (identity and concentrations): Iron: 3600 ppm, Chromium: 14 ppm, Bismuth: 6 ppm, Nickel: 4 ppm
- Other
BET surface area: 23 m²/g, Mean diameter: 70 nm

Test animals

Species

mouse

Strain

other: Crlj:CD1(ICR)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Charles River Laboratories, Japan
- Age at study initiation: 7 weeks
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 21.5-24.1
- Humidity (%): 50.5-64.3
- Photoperiod (hrs dark / hrs light):12:12hrs

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

- Vehicle(s)/solvent(s) used: 0.3% CMC-Na (sodium carboxymethyl cellulose)
- Concentration of test material in vehicle: 0.25, 0.5, 1 mg/mL
- Administration volume: 20 mL/kg/day

Duration of treatment / exposure

2 days

Frequency of treatment

Two times in the interval of 24 hrs (once per day)

Post exposure period

24 hrs

Doses / concentrations

5, 10, 20 mg/kg-bw/day

No. of animals per sex per dose

6

Control animals

yes, concurrent vehicle

Positive control(s)

mitomycin C:

- Route of administration: Oral
- Doses: 5mg/kg-bw
- Administration volume: 20 mL/kg/day
- Dosing time: Once

Examinations**Tissues and cell types examined**

Bone marrow cells, immature erythrocytes

Evaluation criteria

The test was judged positive if a statistically significant increase in the frequency of MNIEs was observed at at least one dose level of MWCNTs, accompanied by a dose-response relationship.

Statistics

Fisher's exact probability test and Dunnett's test.

Any other information on materials and methods incl. tables

The frequency of micronucleated immature erythrocytes (MNIEs) was calculated on the basis of observation of 2000 immature erythrocytes (IEs) per animal. The proportion of IEs among all erythrocytes was determined by counting 1000 erythrocytes for each mouse.

Results and discussions**Test results**

Sex	male/female
Genotoxicity	negative
Toxicity	no effects
Vehicle controls valid	yes
Positive controls valid	yes

Any other information on results incl. tables**Table. Result of bone marrow micronucleus test on MWNT-7 in ICR mice.**

est substance	Dose (mg/kg)	Number of doses	Number of animals	% MNIE (Mean±S.D)
MWNT-7	0	2	6	0.10 ± 0.07
	5	2	6	0.08 ± 0.04
	10	2	6	0.05 ± 0.05
	20	2	6	0.05 ± 0.08
MMC	5	1	6	3.33 ± 2.27*

*MNIE: Micronucleated immature erythrocyte**IE: Immature erythrocyte**MMC: Mitomycin C***: P<0.01, significantly different from negative control (Fisher's exact test).***Overall remarks, attachments****Attached background material****Applicant's summary and conclusion****Interpretation of results**

negative

Conclusions

It was judged that the test substances, MWNT-7, did not increase the micronuclei induction in any dose groups in mice.

Executive summary

The test substances, MWNT-7, did not affect the proportion of immature erythrocytes, the total proportion of erythrocytes, or the number of micronuclei in immature erythrocytes.

Endpoint study record: Nikkiso: Genetic toxicity in vivo.001**Administrative Data**

Purpose flag	key study
Study result type	experimental result Study period Sept. 2010 - Dec. 2010
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to OECD TG 474

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J.	2012	Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of in vitro and in vivo assays.	Regul Toxicol Pharmacol., 63(2):188-195, (2012)					
study report	AIST	2010	Micronucleus assay of Multiwalled carbon Nanotube B (CNT-B) using mice		Ina Research	ZT10127			2010-12-09

Data access

data published

Materials and methods**Type of genotoxicity**

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	

GLP compliance

no

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Nikkiso MWCNTs

Test material form

powder

Details on test material

- Name of test material (as cited in study report): MWNT-7 (Manufactured by Mitsui & Co.,Ltd. (Japan))
- Analytical purity: No data

- Impurities (identity and concentrations): Iron: 3600 ppm, Chromium: 14 ppm, Bismuth: 6 ppm, Nickel: 4 ppm
- Other BET surface area: 23 m²/g, Mean diameter: 70 nm

Test animals

Species

mouse

Strain

other: Crlj:CD1(ICR)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Charles River Laboratories, Japan
- Age at study initiation: 7 weeks
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 21.5-24.1
- Humidity (%): 50.5-64.3
- Photoperiod (hrs dark / hrs light):12:12hrs

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

- Vehicle(s)/solvent(s) used: 0.3% CMC-Na (sodium carboxymethyl cellulose)
- Concentration of test material in vehicle: 0.25, 0.5, 1 mg/mL
- Administration volume: 20 mL/kg/day

Duration of treatment / exposure

2days

Frequency of treatment

Two times in the interval of 24 hrs (once per day)

Post exposure period

24 hrs

Doses / concentrations

5, 10, 20 mg/kg/day

Basis nominal conc.

No. of animals per sex per dose

6

Control animals

yes, concurrent vehicle

Positive control(s)

mitomycin C:

- Route of administration: Oral
- Doses: 5mg/kg-bw
- Administration volume: 20 mL/kg/day
- Dosing time: Once

Examinations**Tissues and cell types examined**

Bone marrow cells, immature erythrocytes

Evaluation criteria

The test was judged positive if a statistically significant increase in the frequency of MNIEs was observed at least one dose level of MWCNTs, accompanied by a dose-response relationship.

Statistics

Fisher's exact probability test and Dunnett's test.

Any other information on materials and methods incl. tables

The frequency of micronucleated immature erythrocytes (MNIEs) was calculated on the basis of observation of 2000 immature erythrocytes (IEs) per animal. The proportion of IEs among all erythrocytes was determined by counting 1000 erythrocytes for each mouse.

Results and discussions**Test results**

Sex	male/female
Genotoxicity	negative
Toxicity	no effects
Vehicle controls valid	yes
Positive controls valid	yes

Additional information on results

The frequencies of micronuclei observed in the test substance groups did not increase dose-dependently

with statistical significance comparing to the vehicle control. Therefore, it was judged to be negative. Additionally, the frequencies of immature erythrocytes in the test substance groups did not reduce with statistical significant difference.

Any other information on results incl. tables

Table. Result of bone marrow micronucleus test on N-MWCNTs in ICR mice.

Test substance	Dose (mg/kg)	Number of doses	Number of animals	% MNIE (Mean
N-MWCNTs	0	2	6	0.11 ± 0.0
	5	2	6	0.18 ± 0.0
	10	2	6	0.13 ± 0.1
	20	2	6	0.13 ± 0.0
MMC	5	1	6	2.73 ± 1.3

MNIE: Micronucleated immature erythrocyte

IE: Immature erythrocyte

MMC: Mitomycin C

***:** $P < 0.01$, significantly different from negative control (Fisher's exact test).

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

It was concluded that the test substance N-MWCNTs did not possess a potency to induce micronuclei and an action of growth inhibition to the bone marrow cells of mice under the present test conditions.

Endpoint study record: Hanwha CM-100: Genetic toxicity in vivo.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to OEC TG 474

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
secondary source	National institute of environmental research, Ministry of Environment, Korea	2009	The production of multi-walled carbon nanotubes(MWCNTs) hazardous data						
publication	Kim JS, Lee K, Lee YH, Cho HS, Kim KH, Choi KH, Lee SH, Song KS, Kang CS, Yu IJ.	2011	Aspect ratio has no effect on genotoxicity of multi-wall carbon nanotubes.	Arch Toxicol., 85: 775-786, (2011)					

Materials and methods**Type of genotoxicity**

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	

GLP compliance

yes

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Hanwha CM-95

Details on test material

- Name of test material (as cited in study report): CM-95 (Manufactured by Hanwha Nanotech Inc. (Korea))
 - Analytical purity: 95%
 - Impurities (identity and concentrations): Iron: approx. 5%
 - Other
- Diameter: 10-15 nm, Length: ~20 µm

Test animals

Species

mouse

Strain

ICR

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: OrientBio, Korea
- Age at study initiation: 8 weeks old
- Weight at study initiation: No data
- Housing: The rats were housed in polycarbonate cages (no more than 3 rats per cage)
- Diet (e.g. ad libitum): ad libitum- Water (e.g. ad libitum): ad libitum
- Acclimation period: 1 week

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 ± 2 °C
- Humidity (%): 55 ± 7 %
- Photoperiod (hrs dark / hrs light): 12 hrs dark/ 12 hrs light

Administration / exposure

Route of administration

intraperitoneal

Vehicle(s)

Vehicle: 1,2-Dipamitoyl-sn-glycero-3-phosphocholine(DPPC)

Post exposure period

24 hrs

Doses / concentrations

12.5, 25 and 50 mg/kg body weigh

No. of animals per sex per dose

Six per dose. In total, thirty six for whole experiments.

Positive control(s)

mitomycin C

Examinations

Tissues and cell types examined

bone marrow erythrocytes in femur.

Details of tissue and slide preparation

Samples were observed under 400 times magnification or more by blind methods with fluorescence microscope and optical microscope. In order to count the number of cells containing micronucleus, around 2,000 polychromatic erythrocytes in good conditions were selected. 200 erythrocytes were selected to determine the presence (or absence) of polychromatic erythrocytes and the ratio between the two was estimated.

Statistics

One way analysis of variance (ANOVA) test

Results and discussions

Test results

Sex male

Genotoxicity negative

Toxicity no effects

Vehicle controls valid yes

Negative controls valid yes

Positive controls valid yes

Additional information on results

Compared with vehicle control counterparts, mice with treated MWCNTs did not exhibit significant body weight changes (Table 5.6). Polychromatic erythrocytes rates in treated mice with MWCNTs did not show significant suppression of proliferation of bone marrow cells compared with untreated control, vehicle control and positive control. (Table 5.7).

Any other information on results incl. tables

Table 5.6. The comparison of body weight for test animal in MWCNTs

Sampling time (hrs)	Groups	Dose (mg/kg)	Animal No.	Body weights (gram, Mean±S.D.)	
				Administration	Sacrifice
24	U.C.	0	6	37.39±1.88	37.05±1.62
	V.C.	0	6	37.54±1.84	37.02±2.00
	MWCNTs	12.5	6	37.06±1.62	37.91±2.17
		25	6	37.62±1.56	37.14±1.74
		50	6	37.55±1.65	37.02±2.02
	MMC	2.0	6	37.68±1.33	37.03±1.39

U.C.: Untreated control, V.C.: Vehicle control, MMC: Mitomycin C(2.0 mg/mL)

Table 5.7. Frequency of PCE/(PCE+NCE) ratio in bone marrow of male mouse treated with indicated doses of MWCNTs for 24 hrs.

Sampling time (hrs)	Groups	Dose (mg/kg)	Animal No.	MNPCE/2000 PCEs(Mean±SD, %)		PCE/(PCE+NCE) (Mean±SD)	
24	U.C.	0	1	0.15	(0.09±0.06)	0.45	(0.53±0.08)
			2	0.05		0.57	
			3	0.00		0.44	
			4	0.10		0.63	
			5	0.15		0.53	
			6	0.10		0.59	
	V.C.	0	7	0.10	(0.08±0.08)	0.52	(0.54±0.10)
			8	0.05		0.57	
			9	0.00		0.61	
			10	0.10		0.57	
			11	0.00		0.35	
			12	0.20		0.63	
	MWCNTs	12.5	13	0.10	(0.13±0.05)	0.55	(0.54±0.05)
			14	0.15		0.49	
			15	0.05		0.52	
			16	0.10		0.50	
			17	0.20		0.56	
			18	0.15		0.63	
		25	19	0.25	(0.15±0.08)	0.40	(0.49±0.07)
			20	0.15		0.56	
			21	0.10		0.54	
			22	0.05		0.57	
			23	0.25		0.44	
			24	0.10		0.47	
		50	25	0.05	(0.11±0.06)	0.52	(0.42±0.08)
			26	0.05		0.47	
			27	0.20		0.49	
			28	0.10		0.29	
			29	0.10		0.38	
			30	0.15		0.39	
	MMC	2.0	31	6.90	(5.70±2.79)*	0.40	(0.43±0.05) *
			32	0.25		0.50	
			33	8.30		0.44	
			34	6.15		0.39	
			35	6.05		0.47	
			36	6.55		0.39	

U.C.: Untreated control, V.C.: Vehicle control, MMC: Mitomycin (2.0 mg/mL), PCE: Polychromatic erythrocytes, NCE: Normochromatic erythrocyte, Vehicle: 1,2-Dipalmitoyl-sn-glycero-3phosphocholine(DPPC)

* significantly different from the vehicle control at $P<0.05$ (One-way ANOVA)

Overall remarks, attachments

Illustration (picture/graph)

To examine the effects of MWCNTs, autopsy was carried out. Transfer of MWCNTs to vessel or bone marrow was not noted (Fig 5.1).



Fig5.1 The picture of MWCNTs (black coloured area) found in mouse abdominal cavity

Applicant's summary and conclusion**Interpretation of results**

negative

Conclusions

There was no statistical difference between the frequencies of MNPCE for MWCNTs treated groups and a vehicle control group.

Endpoint study record: Hanwaha CM-100: Genetic toxicity in vivo.002**Administrative Data**

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to OECD TG 412

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Kim JS, Sung JH, Song KS, Lee JH, Kim SM, Lee GH, Ahn KH, Lee JS, Shin JH, Park JD, Yu IJ.	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., 128:439-448, (2012)					
secondary source	National institute of environmental research, Ministry of Environment, Korea	2009	The production of multi-walled carbon nanotubes (MWCNTs) hazardous data						

Materials and methods**Type of genotoxicity**

DNA damage and/or repair

Type of study

other: in vivocomet assay

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD 412 Inhalation toxicity	

GLP compliance

yes

Test materials

Test material identity

Identifier	Identity
	Hanwha CM-95

Test material form

no data

Details on test material

- Name of test material (as cited in study report): CM-95 (Manufactured by Hanwha Nanotech Inc. (Korea))
 - Analytical purity: >95%
 - Impurities (identity and concentrations): Iron<2%, cobalt<2%, Al₂O₃<4%
 - Other
- Surface area: 224.9 m²/g, Diameter: 10-15 nm, Length: ~20 µm

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: OrientBio, Korea
- Age at study initiation: 8 weeks old
- Weight at study initiation: 273 g
- Housing: The rats were housed in polycarbonate cages (5 rats per cage)
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 1 week

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 21.6 ± 1.2°C
- Humidity (%): 43.7 ± 6.8%
- Photoperiod (hrs dark / hrs light): 12 hrs dark/12 hrs light

Administration / exposure

Route of administration

inhalation

Vehicle(s)

water

Duration of treatment / exposure

6 hr/day, 5 day/week

Post exposure period

1 month and 3 month

Doses / concentrations

0

Basis other: mg/m3

0.16

Basis other: mg/m3

0.34

Basis other: mg/m3

0.94

Basis other: mg/m3

No. of animals per sex per dose

No. of animals per sex per dose: 10

Examinations

Tissues and cell types examined

A single cell gel electrophoresis assay (Comet assay) was conducted to determine the DNA damage in lung cells obtained from the right lung.

Results and discussions

Test results

Sex male

Genotoxicity not determined

Toxicity no effects

Any other information on results incl. tables

The animals exhibited no significant body weight changes, abnormal clinical signs, or mortality during the experiment. As a result, the olive tail moments were 23.00 ± 1.76 , 30.39 ± 1.96 , 22.96 ± 1.26 , and 33.98 ± 2.21

for the control, low-, middle-, and high-concentration groups, respectively, on day 0 post-exposure. Meanwhile, 1 month post-exposure, the olive tail moments were 25.00 ± 2.71 , 28.39 ± 3.55 , 22.56 ± 1.36 , and 31.97 ± 3.16 for the control, low-, middle-, and high-concentration groups, respectively.

Applicant's summary and conclusion

Interpretation of results

other: not determined

Conclusions

The MWCNTs caused a statistically significant increase in lung DNA damage at the high concentration (0.94 mg/m^3) when compared with the negative control group on day 0 and 1 month post-exposure.

7.7 Carcinogenicity

Endpoint study record: Mitsui MWCNT-7: Carcinogenicity.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well-documented publication

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J.	2008	Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube	J. Toxicol. Aci., 33(1), 105-116 (2008)					

Data access

data published

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

GLP compliance

no

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWCNT-7

Details on test material

- Name of test material (as cited in study report): MWCNT
- Lot/batch No.: 060125-01k
- average width: ca 100 nm
- length: 27.5% of the particles were longer than 5 micrometer

Test animals**Species**

mouse

Strain

other: p53-heterozygous (p53(+/-)) mice

Sex

no data

Details on test animals and environmental conditions**TEST ANIMALS**

- Source: (SLC, Shizuoka, Japan)
- Age at study initiation: 9-11 weeks-old
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum

ENVIRONMENTAL CONDITIONS

- Photoperiod (hrs dark / hrs light): 12 hrs dark / 12 hrs light

Administration / exposure**Route of administration**

intraperitoneal

Vehicle

other: 0.5% CMC supplemented with 1.0% Tween 80

Duration of treatment / exposure

single injection

Frequency of treatment

single

Post exposure period

Up to week 25 after instillation

Doses / concentrations

3 mg/head (1×10^9 particles) in 1 ml of suspension

No. of animals per sex per dose

MWCNT group, negative and positive control group: 19 animals per each group

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: The number of the particles was set to a moderate value of the reported ranges (Roller et al., 1997) which corresponds to the maximum value recommended by the draft guideline for man-made mineral fibers (Bernstein and Riego Sintes, 1999).

Positive control

1×10^{10} particles of crocidolite in 1 ml of suspension (corresponds to 3 mg/head)

Examinations

Sacrifice and pathology

At 10 days after injection, the mice were sacrificed and early peritoneal responses were observed microscopically. For evaluation of carcinogenicity, visceral organs including liver, kidney, spleen, lung, digestive tract and macroscopic tumors (en bloc in case of severe peritoneal adhesion) were fixed in 10% neutral buffered formalin. After conventional processing, paraffin-embedded sections were stained with hematoxylin and eosin (H&E) and examined histopathologically under a light microscope. Then, The mice of main groups were monitored until one of the groups reached 100% mortality. The highest lethality was seen in the MWCNT group followed by the Crocidolitegroup, and the study was terminated at week 25 (day 180).

Results and discussions

Results of examinations

Details on results

HISTOPATHOLOGY: NEOPLASTIC

The overall incidence of mesothelioma after the first incidental case found in the MWCNT group at 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 Crocidolite group. Neither tumor induction nor interim death was observed in the Control.

Any other information on results incl. tables

-Early peritoneal responses on day 10 after injection: MWCNT mice showed slight fibrinous adhesion with a trace amount of ascites with scattered black spots of MWCNT aggregates. The intestine loops were edematous and hypotonic. Crocidolite mice showed similar responses but to a lesser extent, and there were no overt peritoneal adhesions. Bluish green spots of crocidolite aggregates were seen on the peritoneal surface.

Applicant's summary and conclusion

Conclusions

In summary, intraperitoneally administered MWCNT has induced mesothelioma in the p53 (+/—) mice carcinogenesis model, probably due to its resemblance to asbestos in size and shape, and biopersistence.

Endpoint study record: Mitsui MWCNT-7: Carcinogenicity.002

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well-documented publication

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Takagi A, Hirose A, Futakuchi M, Tsuda H, Kanno J.	2012	Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice	Cancer Sic., 103 (8), 1440-1444 (2012)					

Data access

data published

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

GLP compliance

no

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWCNT-7

Details on test material

- Name of test material (as cited in study report): MWCNT- Lot/batch No.: 060125-01k

Test animals**Species**

mouse

Strain

other: p53 heterozygous (p53+/-) mouse

Sex

male

Details on test animals and environmental conditions**TEST ANIMALS**

- Source: SLC, Shizuoka, Japan
- Age at study initiation: 9-11 weeks-old
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum

ENVIRONMENTAL CONDITIONS

- Photoperiod (hrs dark / hrs light): 12 hrs dark / 12 hrs light

Administration / exposure**Route of administration**

intraperitoneal

Vehicle

other: 0.5% methyl cellulose supplemented with 1.0% Tween 80

Duration of treatment / exposure

single injection

Frequency of treatment

single exposure

Post exposure period

1 year post exposure

Doses / concentrations

3, 30, 300 µg/mouse (corresponding to 1×10^6 , 1×10^7 , 1×10^8 particles/mouse)

No. of animals per sex per dose

20 mice per dose

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: A previous study showed that micrometer-sized MWCNT (Im-MWCNT) administered intraperitoneally at a dose of 3000 µg/mouse corresponding to 1×10^9 fibers per mouse induced mesotheliomas in p53 heterozygous mice.

Examinations***Sacrifice and pathology***

-Histopathology:Liver, kidney, spleen, lung, digestive tract and macroscopic tumors (en bloc in the case of severe peritoneal adhesion) were fixed in 10% neutral buffered formalin. After conventional processing, paraffin-embedded sections were stained with hematoxylin-eosin (HE) and examined histopathologically under a light microscope. A pair of polarizing filters was set to a light microscope to detect birefringent particles. For the selected atypical mesothelial hyperplasia lesions, serial sections were stained for CD45R(B220), CD3 and F4/80 using anti-mouse CD45R (eBioscience, San Diego, CA, USA), anti-rat CD3 (AbD Serotec, Kidlington, UK), anti-mouse F4/80 antibodies (eBioscience), which were diluted at 1:100, 1:50 and 1:50, respectively.

Results and discussions**Results of examinations*****Details on results*****HISTOPATHOLOGY: NEOPLASTIC**

Peritoneal mesotheliomas were induced in a dose-dependent manner shown by an increase in the cumulative incidence of the tumors. The cumulative incidence of mesotheliomas was 19/20, 17/20 and 5/20, respectively. The severity of peritoneal adhesion and granuloma formation were dose-dependent and minimal in the lowest dose group. However, the time of tumor onset was apparently independent of the dose. 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. Right beneath was a mononuclear cell accumulation consisting of CD45- or CD3-positive lymphocytes and CD45/CD3-negative F4/80 faintly positive macrophages; some of the macrophages contained singular MWCNT in their cytoplasm. The lesions were devoid of epithelioid cell granuloma and fibrosis. These findings were in

favor of the widely proposed mode of action of fiber carcinogenesis, that is, frustrated phagocytosis where the mesotheliomagenic microenvironment on the peritoneal surface is neither qualitatively altered by the density of the fibers per area nor by the formation of granulomas against agglomerates.

Applicant's summary and conclusion

Conclusions

Three groups of p53 heterozygous mice (n = 20) were given a single intraperitoneal injection of 300 ug/mouse of Im-MWCNT (corresponding to 1×10^8 fibers), 30 ug/mouse (1×10^7) or 3 ug/mouse (1×10^6), respectively, and observed for up to 1 year. The cumulative incidence of mesotheliomas was 19/20, 17/20 and 5/20, respectively. The severity of peritoneal adhesion and granuloma formation were dose-dependent and minimal in the lowest dose group. However, the time of tumor onset was apparently independent of the dose.

Endpoint study record: Mitsui MWCNT-7 Carcinogenicity.003

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability	incl. Acceptable, well documented publication which meets basic scientific principles

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Sakamoto Y, Nakae D, Fukumori N, Tayama K, Maekawa A, Imai K, Hirose A, Nishimura T, Ohashi N, Ogata A.	2009	Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats	J Toxicol Sci. 2009;34(1):65-76					

Data access

data published

Materials and methods

GLP compliance

no

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Details on test material

- Name of test material (as cited in study report): MWCNT- Lot/batch No.: 060125-01k- average width: ca 100 nm- length: 27.5% of the particles were longer than 5 micrometer

Test animals**Species**

rat

Strain

other: Fischer 344 DuCr/CrLj

Sex

male

Details on test animals and environmental conditions

A total of 22 male Fischer 344 DuCr/CrLj rats were purchased at their age of 4 weeks old from Charles River Inc. (Kanagawa, Japan). Rats were housed individually in stainless-steel cages (220 x 200 x 160, in millimeter) with wire-netting fronts and floors. The cages were suspended from belt-type racks with an automatic watersupply system providing tap water. The animal room was air-conditioned as 24-25°C, 50-60% relative humidity, and 10 times ventilation per hour using air drawn into the animal room by passing through a filter at the efficiency of 99.9% (HEPA filter). Fluorescent lighting was controlled to give a 12-hr light (6:00-18:00)/dark cycle. After an 8-week acclimation, rats were used for experimentation at their age of 12 weeks old, when the average body weight was 235 g. Animals were given tap water and a CE-2 pellet diet (Clea Japan Inc., Tokyo, Japan) ad libitum, critically monitored to detect any clinical signs and deaths, and weighed weekly throughout the acclimation and experimental periods.

Administration / exposure**Route of administration**

other: intrascrotal injection

Vehicle

CMC (carboxymethyl cellulose)

Details on exposure

Rats at an average body weight of 235 g were administered vehicle (5 animals), crocidolite (10 animals, 2.0 mg/ kg body weight corresponding to 0.47 mg/rat) or MWCNT (7 animals, 1.0 mg/kg body weight corresponding to 0.24 mg/rat) by a single intrascrotal injection for which the anterior skin of the scrotum was surgically incised 2-3 mm in length under the anesthesia with pentobarbital (Nembutal; Dainippon

Sumitomo Pharma Co., Ltd., Osaka, Japan), and suspensions of test chemicals or a vehicle solution were administered into the scrotal cavity at a volume of 2 ml/kg body weight.

Duration of treatment / exposure

a single dose

Post exposure period

Up to 52 weeks

No. of animals per sex per dose

vehicle (5 animals), crocidolite (10 animals) or MWCNT (7 animals)

Control animals

yes, concurrent vehicle

Examinations

Sacrifice and pathology

At autopsy, rats were macroscopically examined throughout the body including celomic cavities. All major organs and tissues, especially tumor tissues of the inner-surface of such cavities and organs suspected to be involved by tumor, were taken, fixed in 10% neutrally buffered formalin, embedded in paraffin and processed by routine hematoxylin and eosin staining for the histological examination.

Statistics

Statistical significance of intergroup difference for the tumor incidence was assessed using Fisher's exact test, and p-values less than 0.05 were considered significant.

Results and discussions

Results of examinations

Clinical signs and mortality

yes

Gross pathology

yes

Histopathology: neoplastic

yes

Details on results

All vehicle- and crocidolite-treated rats healthily survived throughout the 52-week maximal observation period. In contrast, 4 out of 7 MWCNT-treated rats died during weeks 37-40, 2 other rats became moribund at the ends of week 40 and week 50, and only one rat healthily survived until the end of week 52. In dead and moribund rats treated with MWCNT, severe anemia and enlargement of abdomen due to accumulation of ascites were commonly observed, and their body weights were decreased, or in some cases conversely increased due to marked ascites, for several weeks before autopsied. In the MWCNT treatment group, hemorrhagic ascites at an amount of 5-75 ml was present in the abdominal cavity of 4

dead and 2 moribund rats in association with severe fibrous adhesion of organs/tissues and the peritoneum, especially among the diaphragm, liver, stomach, pancreas, spleen and omentum. The liver was strongly deformed, resulting in difficulty to identify lobular segmentation. In such animals, whitish nodules with varied sizes and polypoid or papillary shapes were disseminated throughout the peritoneal wall including that of the scrotal cavity and occupied visceral peritoneum. While the majority of such nodules were small (up to 2 mm in diameter), large tumors (5-20 mm in diameter) were occasionally observed, mostly around the diaphragm and involving the liver, stomach, pancreas, spleen and their surrounding stroma. Abdominal adipose tissues were largely replaced by tumor nodules. In the thoracic cavity, tumor nodules were observed only on the surface of the diaphragm with the exceptions of metastatic lesions detected on the peri- and epicardium detected of 4 dead animals. In the MWCNT-treated rat surviving at the end of week 52, moderate fibrous adhesions and small tumor nodules were observed on the parietal and visceral peritoneum including the wall of the scrotal cavity, but ascites were not present. In addition, small blackcolored spots scattered on the peritoneum of all MWCNT- treated rats. Mesothelial hyperplasias and mesotheliomas were observed in 7 and 6 out of 7 MWCNT-treated rats, respectively. The overall incidence of mesothelioma in MWCNT-treated rats was thus calculated to be 86%, and this value was significantly higher than those of vehicle- or crocidolite-treated rats (both 0%) ($p < 0.05$). In the peritoneum of MWCNT-treated rats, mesothelial cells were generally hypertrophic, and mesothelial hyperplasias and mesotheliomas were frequently observed. Granulomas were found in the celom of both crocidolite and MWCNT-treated, but not vehicle-treated, animals. Fibrously shaped MWCNT particles were found also within organs, for instance in the cytoplasm of portal macrophages and Kupffer cells of the liver, as well as macrophages and multinuclear giant cells of the mesenteric lymph nodes.

Applicant's summary and conclusion

Executive summary

MWCNT was administered 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.

Endpoint study record: Nanocyl NC7000: Carcinogenicity. 001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well-documented publication

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Muller J, Delos M, Panin N, Rabolli V, Huaux F, Lison D.	2009	Absence of Carcinogenic Response to Multiwalled Carbon Nanotubes in a 2-Year Bioassay in the Peritoneal Cavity of the Rat.	Toxicol. Sci., 110(2), 442-448 (2009)					
secondary source	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Data access

data published

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline followed		

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Nanocyl NC7000

Details on test material

MWCNT with structural defects (MWCNT+)MWCNT without structural defects (MWCNT-)

Test animals**Species**

rat

Strain

Wistar

Sex

male

Details on test animals and environmental conditions**TEST ANIMALS**

- Source: Charles River (Brussels, Belgium)
- Age at study initiation: 10-13 weeks-old
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 1 week

ENVIRONMENTAL CONDITIONS

- Temperature (°C): controlled
- Humidity (%): controlled
- Photoperiod (hrs dark / hrs light): 12 hrs dark / 12 hrs light

Administration / exposure**Route of administration**

intraperitoneal

Vehicle

other: phosphate-buffered saline (PBS)

Duration of treatment / exposure

single injection

Frequency of treatment

single

Post exposure period

24 months post-exposure

Doses / concentrations

2 mg/rat (MWCNT+) and 20 mg/rat (MWCNT+, MWCNT-)

No. of animals per sex per dose

MWCNT treated groups: 50 rats per group

Negative and positive control groups: 26 rats per group

Control animals

yes, concurrent vehicle

Positive control

2 mg of crocidolite asbestos

Examinations

Observations and examinations performed and frequency

Body weight and survival rate were traced.

Sacrifice and pathology

Histopathology: Peritoneal tumors were investigated. The incidence of mesothelioma and other tumors was recorded.

Statistics

The incidence of tumors across the different groups, except asbestos positive controls, was compared with the Fisher exact test. Statistical significance was set at $p < 0.05$.

Results and discussions

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Histopathology: neoplastic

yes

Details on results

HISTOPATHOLOGY: NEOPLASTIC Although crocidolite (positive control) induced a clear carcinogenic response (34.6% animals with mesothelioma vs. 3.8% in vehicle controls), MWCNT with or without structural defects did not induce mesothelioma in this bioassay (4, 0, or 6%, respectively). The incidence of tumors other than mesothelioma was not significantly increased across the groups.

Applicant's summary and conclusion

Conclusions

The incidence of mesothelioma and other tumors was recorded in three groups of 50 male Wistar rats injected intraperitoneally with a single dose of MWCNT with defects (2 or 20 mg/animal) and MWCNT without defects (20 mg/animal). Two additional groups of 26 rats were used as positive (2 mg UICC crocidolite/animal) and vehicle controls. After 24 months, although crocidolite induced a clear carcinogenic response (34.6% animals with mesothelioma vs. 3.8% in vehicle controls), MWCNT with or without structural defects did not induce mesothelioma in this bioassay (4, 0, or 6%, respectively). The incidence of tumors other than mesothelioma was not significantly increased across the groups.

7.8 Toxicity to reproduction

7.8.1 Toxicity to reproduction

7.8.2 Developmental toxicity / teratogenicity

Endpoint study record: Developmental toxicity / teratogenicity.001

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability incl. Acceptable, well-documented publication which meets basic deficiencies scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Fujitani, T. et al.	2012	Teratogenicity of multi-wall carbon nanotube (MWCNT) in ICR mice.	J. Txicol. Sci., vol. 37 (No. 1), 81-89.					

Data access

data published

Materials and methods

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

Teratogenicity study by single intratracheal or intraperitoneal prenatal administration.

GLP compliance

no

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Mitsui MWCNT-7

Details on test material

- Name of test material (as cited in study report): MWCNT
- Lot/batch No.: 060125-01K

Test animals**Species**

mouse

Strain

ICR

Details on test animals and environmental conditions**TEST ANIMALS**

- Source: Charles River Japan Inc., Kanagawa, Japan.
- Age at study initiation: 5 weeks-old (at arrival); 8- 13 weeks old (at mating)
- Weight at study initiation:
- Fasting period before study:
- Housing: housed individually in plastic cage (180 X 305 X 110 mm³) with cedar chip bedding.
- Diet (e.g. ad libitum): standard diet CE2 (Nihon Clea, Inc., Tokyo, Japan), ad libitum.
- Water (e.g. ad libitum): water, ad libitum.
- Acclimation period: not stated.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23- 25 °C
- Humidity (%): 50-60 %
- Air changes (per hr): 10 ventilation per hour
- Photoperiod (hrs dark / hrs light): 12 hrs dark / 12 hrs light

Administration / exposure**Route of administration**

other: intratracheal or intraperitoneal

Vehicle

CMC (carboxymethyl cellulose) (2% CMC-Na solution)

Details on exposure**PREPARATION OF DOSING SOLUTIONS:**

MWCNT was suspended in 2% CMC-Na solution at concentrations of 0.2, 0.3, 0.4 or 0.5 mg/ml for the intraperitoneal study to achieve a uniform administration volume of 10 mL/kg body weight. In the case of the intratracheal study, 3, 4 and 5 mg/ml suspensions were prepared to achieve a uniform administration volume of 1 mL/kg. The control (0 mg/kg bw) animals received 2% CMC-Na solution, intraperitoneally or intratracheally, respectively. These suspensions as well as a vehicle 2% CMC-Na solution were sterilized by an autoclave at 120°C for 20 min and vigorously mixed by hand shaking immediately prior to the administration.

VEHICLE

- Justification for use and choice of vehicle (if other than water): selected as likely as previous studies with the same MWCNTs.
- Concentration in vehicle: 0.2, 0.3, 0.4 and 0.5 mg/ml (intraperitoneal administration); 3, 4 and 5 mg/ml (intratracheal administration)
- Amount of vehicle (if gavage): 10 mL/kg bw (intraperitoneal administration); 1 mL/kg bw (intratracheal administration)
- Purity:

Analytical verification of doses or concentrations

no

Details on mating procedure

- Impregnation procedure: cohoused
- M/F ratio per cage: 1:1
- Length of cohabitation: One overnight
- Proof of pregnancy: sperm in vaginal smear referred to as day 0 of pregnancy

Duration of treatment / exposure

Single exposure on GD (gestational day) 9.

Frequency of treatment

Single

Duration of test

Until GD 18 when the animals were sacrificed.

Doses / concentrations

2, 3, 4 and 5 mg/kg bw (intraperitoneal administration)

Basis actual ingested

3, 4 and 5 mg/kg bw (intratracheal administration)

Basis actual ingested

No. of animals per sex per dose

Experiment 1: 8-13 pregnant females per group (intraperitoneal administration)

Experiment 2: 5-15 pregnant females per group (intratracheal administration)

Control animals

yes, concurrent vehicle

Examinations

Maternal examinations

CAGE SIDE OBSERVATIONS: Yes

- Time schedule: daily during test period

DETAILED CLINICAL OBSERVATIONS: No

BODY WEIGHT: Yes

- Time schedule for examinations: daily during test period

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): No

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): No

POST-MORTEM EXAMINATIONS: Yes

- Sacrifice on gestation day 18 under light ether anesthesia.

- Organs examined: The liver, lung, spleen, heart, kidney, thymus and tracheobronchial lymph node of each dam were removed and weighed.

OTHER:HAEMATOLOGY:

- Parameters examined: leukocyte count, leukocyte subtypes

Ovaries and uterine content

The ovaries and uterine content was examined after termination: Yes

Examinations included:

- Gravid uterus weight: No

- Number of corpora lutea: Yes

- Number of implantations: Yes

- Number of early resorptions: Yes

- Number of late resorptions: Yes

- Other: Number and body weight of live fetuses

Fetal examinations

- External examinations: Yes: [all per litter]

- Soft tissue examinations: No

- Skeletal examinations: Yes: [all per litter]

- Head examinations: No

Statistics

Scheffe's multiple comparison was applied for the organ weights of dams, maternal body weights, number of implantations and live fetuses, and fetal body weights. The incidence of pregnant females and of litters with malformed fetuses, and the number of malformed fetuses were analyzed using Chi square test. The rank sum test was used for data on the resorption and the percent incidence of malformations. The trend test (cumulative X2 test) was performed to evaluate the significance of the development of malformations by the administered doses of MWCNT.

Results and discussions

Maternal toxic effects

yes (Effects were seen in body weight, organ weight, haematology and/or uterine contents; intraperitoneal route (chiefly 4- and 5- mg/kg bw group; some in all treatment groups) and intratracheal route (5-mg/kg bw group).)

Details on maternal toxic effects

EXPERIMENT 1: intraperitoneal administration

- Final body weight (on GD18): Body weights in dams on GD18 were significantly decreased in 4- and 5-mg/kg bw group (Table 1).
- Organ weights: Absolute weight of the spleen revealed significant decreases in all treatment groups (Table 1).
- Haematology: Increases in neutrophil and eosinophil counts were significantly found in all treatment groups except for 5-mg/kgbw group (Table 1).
- Number of females with >1 live fetuses: The number of females with >1 live fetuses were significantly decreased in 4- and 5-mg/kg bw group (Table 2).
- Uterine contents: The rates of early resorptions of fetuses were significantly increased in 4- and 5-mg/kg bw group (Table 2).

EXPERIMENT 2: intratracheal administration

- Final body weight (on GD18): Body weights in dams on GD18 were significantly decreased in 5-mg/kg bw group (Table 4).
- Organ weights: Absolute weight of the lung revealed significant increase in 5-mg/kg bw group (Table 4).
- Haematology: Total leukocyte counts were significantly increased in 4- and 5-mg/kg bw group (Table 4).
- Uterine contents: No treatment-related effects were seen in the numbers of corpora lutea, implantations or the rates of resorptions (Table 5).

Embryotoxic / teratogenic effects

yes (Effects were seen in number of live fetuses and fetal body weight, and incidences of external and skeletal malformations; intraperitoneal route (malformations observed in all treatment groups) vs intratracheal route (malformations seen in 4- and 5-mg/kg).)

Details on embryotoxic / teratogenic effects

EXPERIMENT 1: intraperitoneal administration

- Number and body weights of live fetuses: The number of live fetuses per litter significantly decreased in 4- and 5-mg/kg bw. Body weights of live fetuses revealed significant decreases in all treatment groups of both sexes except for 5-mg/kg bw group (Table 2).
- External malformations: The number of malformed fetuses/examined were significantly increased in all treatment groups. Malformations such as short or absent tail, cleft palate or reduction of deformity of limb were not observed in the control group (Table 3).
- Skeletal malformations: Both the number of litters with malformed fetuses/examined and the number of malformed fetuses/examined were significantly increased in all treatment groups. Various malformations such as fusions of ribs, fusions of vertebral bodies and arches, hypophalangia or hyperphalangia were not observed in the control group (Table 3).

EXPERIMENT 2: intratracheal administration

- Number and body weights of live fetuses: Body weights of live fetuses decreased significantly in both sexes of 5-mg/kg bw group (Table 5).
- External malformations: Both the number of litters with malformed fetuses/examined and the number of

malformed fetuses/examined were significantly increased in 4- and 5- mg/kg bw group. The number of fetuses with short or absent tail, and with reduction of deformity of limb decreased significantly in these groups (Table 6).

- Skeletal malformations: Both the number of litters with malformed fetuses/examined and the number of malformed fetuses/examined were significantly increased in 4- and 5-mg/kg bw group. The number of fetuses with fusions of ribs as well as fusions of vertebral bodies and arches were significantly increased in these groups, and the number of fetuses with hypophalangia in 4-mg/kg bw group (Table 6).

Any other information on results incl. tables

Table 1 Body and organ weights, and leukocyte typing and counting in pregnant mice treated with single intraperitoneal administration of MWCNT (Experiment 1)

Items	MWCNT dose (mg/kg body weight)				
	0 (control)	2	3	4	5
Number of dam	10	8	9	13	9
Body weight on day 9 of the gestation	36.1 ± 1.3	35.3 ± 1.9	35.2 ± 2.9	35.2 ± 2.6	34.6 ± 2.4
Body weight on day 18 of the gestation	62.4 ± 2.8	55.7 ± 12.0	49.2 ± 7.9	46.3 ± 11.9*	38.8 ± 9.4***
Organ weight					
-Liver (g)	3.11 ± 0.40	3.17 ± 0.53	2.99 ± 0.49	2.80 ± 0.52	2.42 ± 0.64
-Kidney (mg)	478 ± 133	503 ± 52	447 ± 48	472 ± 55	452 ± 55
-Heart (mg)	179 ± 17	180 ± 23	167 ± 18	165 ± 16	157 ± 22
-Lung (mg)	189 ± 8	181 ± 3	176 ± 20	188 ± 15	202 ± 20
-Spleen (mg)	145 ± 40	297 ± 88*	323 ± 86**	333 ± 99**	372 ± 91***
-Thymus (mg)	26.6 ± 12.9	22.6 ± 5.4	17.2 ± 9.2	25.3 ± 7.4	37.8 ± 15.0
-Tracheobronchial lymph node (mg)	7.4 ± 8.3	7.3 ± 3.5	8.8 ± 4.5	6.2 ± 4.5	14.6 ± 5.1
Leukocyte count (10 ² /μl)					
-Total	47 ± 19	115 ± 34	124 ± 48*	109 ± 68	82 ± 38
-Lymphocyte	28.9 ± 11.7	38.8 ± 12.0	33.6 ± 15.1	33.0 ± 35.7	23.9 ± 11.4
-Neutrophil	15.0 ± 7.0	54.6 ± 19.2*	66.1 ± 23.0**	53.6 ± 37.0*	46.5 ± 23.9
-Eosinophil	0.9 ± 0.5	17.5 ± 14.1**	16.1 ± 8.9**	15.5 ± 8.1**	6.7 ± 5.0
-Monocyte	1.9 ± 1.7	4.1 ± 3.0	8.2 ± 7.8	7.0 ± 4.2	5.5 ± 4.0

Values are the mean ± S.D. Asterisks represent that the values are significantly different from the control value (*, ** or *** indicating $p < 0.05$, 0.01 or 0.001 , respectively).

Table 2 Reproductive data of pregnant mice treated with single intraperitoneal administration of MWCNT (Experiment 1)

Reproductive parameters	MWCNT dose (mg/kg body weight)				
	0 (control)	2	3	4	5
Female mated ¹⁾	11	12	12	15	10
Female died ²⁾	0	0	0	0	0

Female gestated ³⁾	10	8	9	13	9
Female with >1 live fetus	10	7	8	7*	3**
Corpora lutea/litter [#]	15.8 ± 1.9	15.6 ± 1.6	16.0 ± 4.1	15.4 ± 1.8	14.4 ± 2.2
Implantations/litter [#]	14.5 ± 2.5	14.4 ± 1.5	12.3 ± 2.7	14.0 ± 2.1	12.7 ± 3.8
Resorption of fetuses (%) ⁴⁾ [#]					
-Early	11.0 ± 13.5	35.3 ± 34.9	41.7 ± 34.8	67.1 ± 38.8**	81.7 ± 28.2**
-Late	1.7 ± 3.7	2.4 ± 3.4	0	1.6 ± 3.1	0.9 ± 2.6
Live fetus/litter [#]	12.6 ± 2.6	9.5 ± 5.1	7.3 ± 4.1	4.8 ± 5.8**	1.4 ± 3.3***
Body weight of live fetus (g) [#]					
-Male	1.48 ± 0.10	1.29 ± 0.08*	1.28 ± 0.10**	1.31 ± 0.08*	1.42 ± 0.12
-Female	1.43 ± 0.13	1.23 ± 0.09*	1.24 ± 0.12*	1.21 ± 0.11*	1.33 ± 0.02

1) Number of animals with vaginal plug. 2) Number of animals died before the scheduled sacrifice on day 18 of the gestation. 3) Number of animals with implantation sites. 4) 'Early' was defined as a case showing the implanted sites and amorphous mass, while 'Late' was defined as a case showing the head and limbs.

[#]: Values are the means ± S.D. The present resorption and foetal body weight were obtained by averaging the value of each litter. Asterisks represent that the values are significantly different from the control value (*, ** or *** indicating $p < 0.05$, 0.01 or 0.001 , respectively).

Table 3 Incidences of malformations in fetuses born from pregnant mice treated with single intraperitoneal administration of MWCNT (Experiment 1)

Items	MWCNT dose (mg/kg body weight)				5
	0 (control)	2	3	4	
External malformation					
Numbers of litters with malformed fetuses/examined (percentages in the parentheses)	0/10 (0)	2/7 (28.6)	2/8 (25.0)	3/7 (42.9)*	1/3 (33.3)
Percent incidence of malformations [#]	0	9.2 ± 18.8	3.6 ± 6.8	4.6 ± 6.5	6.7 ± 11.5
Numbers of malformed fetuses/examined	0/126	3/76*	3/66*	3/63*	2/13***
Numbers of fetuses with short or absent tail	0	2	1	1	0
Numbers of fetuses with cleft palate	0	0	0	1	0
Numbers of fetuses with reduction deformity of limb	0	2	3	1	2
Skeletal malformation					
Numbers of litters with malformed fetuses/examined (percentages in the parentheses)	0/10 (0)	4/7 (57.1)**	3/8 (37.5)*	3/7 (42.9)*	2/3 (66.7)**
Percent incidence of malformations [#]	0.0 ± 0.0	14.4 ± 18.1	11.1 ± 21.7	11.9 ± 19.2	40.0 ± 52.9
Numbers of malformed fetuses/examined	0/126	9/76***	7/66***	7/63***	5/13***

Numbers of fetuses with fusion of ribs	0	3	1	2	0
Numbers of fetuses with vertebral bodies and arches	0	6	7	0	3
Numbers of fetuses with hypophalangia	0	2	2	3	2
Numbers of fetuses with hyperphalangia	0	0	0	2	0

#: Calculated by averaging the percentage in each litter (i.e., numbers of malformations/fetuses) and shown as the means $\pm$ S.D.

Asterisks represent that the values are significantly different from the control value (*, ** or *** indicating $p < 0.05$, 0.01 or 0.001 , respectively).

Table 4 Body and organ weights, and leukocyte typing and counting in pregnant mice treated with single intratracheal administration of MWCNT (Experiment 2)

Items	MWCNT dose (mg/kg body weight)			
	0 (control)	3	4	5
Number of dam	10	10	15	5
Body weight on day 9 of the gestation	33.0 $\pm$ 2.0	33.6 $\pm$ 2.8	33.8 $\pm$ 2.8	32.5 $\pm$ 2.2
Body weight on day 18 of the gestation	55.4 $\pm$ 3.1	58.4 $\pm$ 5.5	59.1 $\pm$ 6.9	45.1 $\pm$ 4.5*
Organ weight				
-Liver (g)	2.80 $\pm$ 0.27	2.73 $\pm$ 0.36	3.05 $\pm$ 0.31	2.44 $\pm$ 0.11
-Kidney (mg)	454 $\pm$ 52	431 $\pm$ 60	457 $\pm$ 54	422 $\pm$ 28
-Heart (mg)	155 $\pm$ 10	161 $\pm$ 14	162 $\pm$ 15	150 $\pm$ 9
-Lung (mg)	157 $\pm$ 14	168 $\pm$ 10	197 $\pm$ 51	228 $\pm$ 47**
-Spleen (mg)	136 $\pm$ 22	122 $\pm$ 29	149 $\pm$ 40	158 $\pm$ 35
-Thymus (mg)	19.9 $\pm$ 7.5	16.4 $\pm$ 5.3	18.9 $\pm$ 5.5	13.9 $\pm$ 8.9
-Tracheobronchial lymph node (mg)	4.2 $\pm$ 3.6	6.8 $\pm$ 5.2	6.2 $\pm$ 4.3	8.7 $\pm$ 2.3
Leukocyte count (10 ² /μl)				
-Total	37.5 $\pm$ 6.4	49.5 $\pm$ 11.3	51.6 $\pm$ 11.5*	51.3 $\pm$ 10.6*
-Lymphocyte	21.0 $\pm$ 4.4	30.0 $\pm$ 8.2	26.5 $\pm$ 7.4	22.5 $\pm$ 6.4
-Neutrophil	14.7 $\pm$ 4.5	17.4 $\pm$ 9.7	20.3 $\pm$ 11.2	25.4 $\pm$ 11.5
-Eosinophil	0.7 $\pm$ 0.9	1.1 $\pm$ 0.7	2.7 $\pm$ 2.5	1.6 $\pm$ 1.1
-Monocyte	1.2 $\pm$ 0.7	1.0 $\pm$ 0.5	2.2 $\pm$ 1.4	1.7 $\pm$ 0.4

Values are the means $\pm$ S.D. Asterisks represent that values are significantly different from the control value (* or ** indicating $p < 0.05$ or 0.01 , respectively).

Table 5 Reproductive data of pregnant mice treated with single intratracheal administration of MWCNT (Experiment 2)

Reproductive parameters	MWCNT dose (mg/kg body weight)			
	0 (control)	3	4	5
Female mated ¹⁾	11	12	16	6
Female died ²⁾	0	0	0	0
Female gestated ³⁾	10	10	15	5

Female with >1 live fetus	10	10	15	5
Corpora lutea/litter [#]	14.6 ± 1.5	16.0 ± 1.8	15.1 ± 1.8	15.8 ± 2.3
Implantations/litter [#]	12.8 ± 1.6	14.8 ± 2.2	13.8 ± 2.7	11.8 ± 2.9
Resorption of fetuses (%) ^{4) #}				
-Early	9.8 ± 13.4	8.8 ± 8.4	21.0 ± 29.8	20.0 ± 17.7
-Late	2.0 ± 4.6	0.6 ± 1.8	0.8 ± 2.2	6.3 ± 10.1
Live fetus/litter [#]	11.3 ± 2.1	13.3 ± 1.5	10.5 ± 4.4	8.8 ± 2.9
Body weight of live fetus (g) [#]				
-Male	1.41 ± 0.14	1.36 ± 0.12	1.23 ± 0.19	1.07 ± 0.20*
-Female	1.35 ± 0.13	1.31 ± 0.11	1.19 ± 0.19	1.06 ± 0.18*

1) Number of animals with vaginal plug. 2) Number of animals died before scheduled sacrifice on day 18 of the gestation. 3) Number of animals with implantation sites. 4) 'Early' was defined as a case showing the implanted sites and amorphous mass, while 'Late' was defined as a case showing the head and limbs.

#: Values are the means ± S.D. The percent resorption and fetal body weight were obtained by averaging the value for each litter. Asterisks represent that the values are significantly different from the control value (* indicating $p < 0.05$).

Table 6 Incidences of malformations in fetuses from pregnant mice treated with single intratracheal administration of MWCNT (Experiment 2)

Experiment 2)

Items	MWCNT dose (mg/kg body weight)			
	0 (control)	3	4	5
External malformation				
Numbers of litters with malformed fetuses/examined (percentages in the parentheses)	0/10 (0)	0/10 (0)	5/14 (35.7)*	2/5 (40.0)*
Percent incidence of malformations [#]	0	0	15.6 ± 27.9	5.6 ± 8.2
Numbers of malformed fetuses/examined	0/113	0/133	15/158***	3/44**
Numbers of fetuses with short or absent tail	0	0	12**	3**
Numbers of fetuses with reduction deformity of limb	0	0	7*	0
Skeletal malformation				
Numbers of litters with malformed fetuses/examined (percentages in the parentheses)	0/10 (0)	1/10 (10.0)	6/14 (42.8)*	4/5 (80.0)*
Percent incidence of malformations [#]	0	0	39.9 ± 48.4*	61.9 ± 38.2*
Numbers of malformed fetuses/examined	0/113	1/133	56/158***	31/44***
Numbers of fetuses with fusion of ribs	0	0	8*	10***
Numbers of fetuses with vertebral bodies and arches	0	0	54***	25***

Numbers of fetuses with hypophalangia	0	0	10*	1
Numbers of fetuses with hyperphalangia	0	1	0	1

#: Calculated by averaging the percentage in each litter (i.e., number of malformations/fetuses) and shown as the means $\pm$ S.D.

Asterisks represent that the values are significantly different from the control value (*, ** or *** indicating $p < 0.05$, 0.01 or 0.001 , respectively).

Applicant's summary and conclusion

Conclusions

A possible teratogenicity of multi-wall carbon nanotube (MWCNT) was assessed using ICR mice. MWCNTs were suspended in 2% carboxymethyl cellulose and given intraperitoneally or intratracheally to pregnant ICR mice on day 9 of gestation. All fetuses were removed from the uterus on day 18 of gestation, and were examined for external and skeletal anomalies. In the intraperitoneal study, various types of malformations were observed in all MWCNT-treated groups (2, 3, 4 and 5 mg/kg bw). In contrast, 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 malformations and that of litters having fetuses with skeletal malformations were both increased in proportion to the doses of MWCNT. The results showed that MWCNT has a potency of teratogenicity at least under the present experimental conditions.

Endpoint study record: Developmental toxicity / teratogenicity.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl.	Acceptable, well-documented publication which meets basic scientific principles.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Lim, J-H. et al.	2011	Maternal exposure to multi-wall carbon nanotubes dose not induce embryo-fetal developmental toxicity in rats.	Birth Defects Research (Part B), 92, 69-76.					
publication	Lim, H-J. et al.	2011	Evaluation of maternal toxicity in rats exposed to multi-wall carbon nanotubes during pregnancy.	Environmental Health and Toxicology, vol. 26, Article ID: e2011006, published online: Apr. 14, 2011. This article is available from: http://e-ehp.org/					

Data access

data published

Materials and methods**Limit test**

no

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 414 (Prenatal Developmental Toxicity Study)	yes (less number of dams per group)

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Hanwha CM-95

Details on test material

- Name of test material (as cited in study report): MWCNT (Multi-wall carbon nanotubes)
- Analytical purity: 95%
- Other: CM-95 (CM-100 related substance), 10-15 nm in diameter, > 20 µm in length.

Test animals**Species**

rat

Strain

Sprague-Dawley

Details on test animals and environmental conditions**TEST ANIMALS**

- Source: Orient Bio (Seoul, Republic of Korea)
- Age at study initiation: 11 weeks-old- Weight at study initiation:
- Housing: For mating, two females were cohabited with one male in a cage overnight. The mated females were housed individually in polycarbonate cages.
- Diet (e.g. ad libitum): commercial rodent chow, ad libitum
- Water (e.g. ad libitum): sterilized tap water, ad libitum.
- Acclimation period: one week including quarantine.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 ± 3 °C
- Humidity (%): 50 ± 10 %
- Air changes (per hr): 13 to 18 air changes per hour- Photoperiod (hrs dark / hrs light): 12 hrs dark / 12 hrs light

Administration / exposure**Route of administration**

oral: gavage

Vehicle

CMC (carboxymethyl cellulose) (1% CMC-Na aqueous solution)

Details on exposure**PREPARATION OF DOSING SOLUTIONS:**

The test substance was suspended in a 1% CMC-Na vehicle. The suspension was subjected to ultrasonication for 3 minutes to obtain a more homogenous and dispersed suspension. The test mixture was prepared daily prior to use, and the 1% CMC solution alone was used as the vehicle control.

VEHICLE

- Concentration in vehicle: 0.4, 2, 10 and 50 mg/mL
- Amount of vehicle (if gavage): 20 mL/kg/day

Analytical verification of doses or concentrations

no data

Details on mating procedure

- Impregnation procedure: cohoused- M/F ratio per cage: 1:2
- Length of cohabitation: not stated.
- Proof of pregnancy: sperm in vaginal smear referred to as day 0 of pregnancy (GD 0).

Duration of treatment / exposure

14 days from GD 6 through GD19.

Frequency of treatment

once daily.

Duration of test

until GD20 when all pregnant females were sacrificed.

Doses / concentrations

8, 40, 200 and 1,000 mg/kg bw/day

Basis actual ingested

No. of animals per sex per dose

12 pregnant females per dose.

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale: As a preliminary dose-range finding study, five females per group were orally administered at doses of 10, 100 and 1,000 mg/kg bw/day for 10 days. Maternal toxicity and developmental toxicity was not observed even in the high-dose group. Therefore, the highest dose was set as a limit dose of 1,000 mg/kg bw/day (defined by OECD test guideline), and using the common ration of 5, high, middle and low doses were selected as 200, 40 and 8 mg/kg bw/day, respectively.

Examinations***Maternal examinations***

CAGE SIDE OBSERVATIONS: Yes

- Time schedule: daily during the test period.- Cage side observations checked were included: mortality, morbidity, general appearance and behavior.

DETAILED CLINICAL OBSERVATIONS: No

BODY WEIGHT: Yes

- Time schedule for examinations: GD 0, 6, 9, 12, 15, and 19.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Yes

- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

- Time schedule for examinations: GD 0, 6, 9, 12, 15, and 19.

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): No

POST-MORTEM EXAMINATIONS: Yes

- Sacrifice on gestation day 20.

- Organs examined: A complete gross postmortem examination was performed. The absolute and relative weights of the lungs, adrenal glands, liver, spleen, kidneys, thymus, heart, and ovaries were recorded.

OTHER:

SERUM BIOCHEMICAL EXAMINATIONS:

- Collection of blood: blood samples were taken from posterior vena cava.
 - Parameters examined: aspartate aminotransferase, alanine aminotransferase, total cholesterol, triglyceride, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, total bilirubin, total protein and albumin.

ANALYSIS OF KIDNEY OXIDATION DAMAGE:

- Preparation of kidney: After crushing the kidney tissue using a glass-teflon homogenous grinder for a few seconds, the smashed tissue was centrifuged at 4°C, at 800 g for 10 minutes. The protein was quantified according to the Lowry method. Activity of antioxidant enzymes (catalase, glutathione reductase, glutathione peroxidase, glutathione-S-transferase), glutathione concentration, and degree of lipid peroxidation were also measured.

Ovaries and uterine content

The ovaries and uterine content was examined after termination: Yes

Examinations included:

- Gravid uterus weight: Yes
- Number of corpora lutea: Yes
- Number of implantations: Yes
- Number of early resorptions: Yes
- Number of late resorptions: Yes
- Other: live and dead fetuses were recorded.

Fetal examinations

- External examinations: Yes: [all per litter]
- Soft tissue examinations: Yes: [half per litter]
- Skeletal examinations: Yes: [half per litter]
- Head examinations: No
- Other: Fetal weight and placental weight were recorded. Sex ratio of fetuses was calculated.

Statistics

Data were presented as means $\pm$ S.D. The unit of statistical measurement was expressed as per the pregnant female or the litter. Quantitative data, such as maternal body weight, food consumption, fetal body weight, and placental weight, were subjected to a one-way analysis of variance. Scheffe's multiple comparison test was carried out when the differences were significant. The number of corpora lutea, total implantations, live and dead fetuses, and fetal alterations was evaluated statistically using Kruskal-Wallis nonparametric analysis of variance, followed by the Mann-Whitney U-test where appropriate. The gender ratio and proportions of litters with malformations and developmental variations were compared using chi-square tests and Fisher's exact probability tests. GraphPad InStat version 3.0 (GraphPad Software Inc., CA, USA) was used for statistical analysis of control and treatment groups. A P value of 0.05 or lower was considered to be statistically significant.

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Based on	Basis for effect level / Remarks
NOAEL	maternal toxicity	200 mg/kg bw/day (nominal)	test mat.	Overall effects.
LOAEL	maternal toxicity	1000 mg/kg bw/day (nominal)	test mat.	Decrease in thymus weight in 1000 mg/kg bw day group.
NOAEL	developmental toxicity	1000 mg/kg bw/day (nominal)	test mat.	No adverse effects on embryotoxicity or on teratogenicity up to 1000 mg/kg bw/day.

Maternal toxic effects

yes (1,000 mg/kg bw/day: decrease in thymus weight)

Details on maternal toxic effects

Clinical signs: Decreased locomotor activity and depression were observed in one dam of the 40 mg/kg group, and 3 dams of the 1,000 mg/kg group. These findings were transiently observed after treatment and were not dose-dependent in either incidence or severity, and thus considered by the author as not treatment-related. Body weight change: No effects observed (Table 1).

Food consumption: No treatment-related effects observed. Gravid uterine weight: No effects observed (Table 1).

Organ weights: There were significant decreases in absolute and relative weights of the thymus in the 1000 mg/kg group, while other organ weights in the treatment groups did not show significant difference compared to the control group. Decrease in thymus weight was considered as treatment-related effect (Table 2).

Serum biochemical examinations: No effects observed (Table 3).

Parameters on kidney oxidation damage: No effects observed.

Uterine contents: The number of corpora lutea, implantations, pre and postimplantation loss rates, fetal deaths, litter size were similar in the treatment groups and the control group (Table 4).

Embryotoxic / teratogenic effects

no effects

Details on embryotoxic / teratogenic effects

Sex ratio and fetal weight: No significant differences between the control and treatment groups (Table 4).

Fetal malformations: No external and visceral malformations were observed in any of the groups. Visceral variations, including misshapen thymus and dilated ureter, were observed, however, incidences of these findings were comparable between the groups (Table 5). No skeletal malformations were observed in any of the groups. Although there were some types of skeletal variations including enlarged fontanel, incomplete ossification of interparietal, bipartite ossification of sternbrae, misshapen sternbrae, short supernumerary ribs, dumbbell ossification of thoracic centrum, bipartite ossification of lumbar centrum, supernumerary lumbar centrum, incomplete ossification of pubis, and incomplete ossification of ischium, there were no significant differences in the number of fetuses with developmental variations or in the number of litters with affected fetuses between the groups. In addition, there was no evidence of exposure-related reductions in ossification of fetal skeletons (Table 6).

Any other information on results incl. tables

Table 1 Body weight changes in pregnant rats treated with MWCNT during gestation days 6 -19

Parameters	Multi-wall carbon nanotubes (mg/kg/d)			
	0	8	40	
No. of rats mated	12	12	12	
No. of pregnant rats	11	9	10	
Gestational day 0	246.5 ± 11.14	247.5 ± 11.90	247.1 ± 17.08	24
Gestational day 6	283.7 ± 12.60	284.6 ± 15.97	286.1 ± 19.65	28
Gestational day 9	296.2 ± 15.63	293.5 ± 16.38	297.3 ± 20.35	28
Gestational day 12	309.8 ± 15.56	309.2 ± 13.97	312.5 ± 20.66	30
Gestational day 15	323.0 ± 16.30	324.2 ± 12.88	328.6 ± 21.05	31
Gestational day 20	381.3 ± 25.97	386.6 ± 13.88	387.4 ± 28.61	36
Body weight gain during pregnancy	134.8 ± 17.12	139.1 ± 14.79	140.3 ± 21.11	12
Corrected body weight ^a	319.9 ± 16.77	317.3 ± 16.92	323.4 ± 23.07	30
Gravid uterine weight	61.4 ± 16.72	69.3 ± 9.49	63.9 ± 21.99	6

Values are presented as means ± SD (g).

a: Body weight on gestational day 20 - gravid uterine weight.

Table 2 Absolute and relative organ weights of pregnant rats treated with MWCNT during gestational days 6-19

Parameters	Multi-wall carbon nanotubes (mg/kg/day)				
	0	8	40	200	1,000
No. of dams	11	9	10	12	11
Body weight at term	381.3 ± 25.97	386.6 ± 13.88	387.4 ± 28.61	367.2 ± 28.13	377.4 ± 39.56
Lung (g)	1.25 ± 0.122	1.30 ± 0.153	1.24 ± 0.083	1.24 ± 0.106	1.20 ± 0.090
Per body weight (%)	0.33 ± 0.029	0.34 ± 0.040	0.32 ± 0.021	0.34 ± 0.035	0.32 ± 0.021
Adrenal glands (g)	0.087 ± 0.0078	0.079 ± 0.0137	0.076 ± 0.0127	0.089 ± 0.0142	0.087 ± 0.0143
Per body weight (%)	0.023 ± 0.0017	0.020 ± 0.0037	0.020 ± 0.0028	0.024 ± 0.0048	0.023 ± 0.0056
Liver (g)	15.39 ± 1.673	15.45 ± 0.862	15.50 ± 1.661	15.08 ± 1.964	15.05 ± 1.855
Per body weight (%)	4.03 ± 0.245	4.00 ± 0.161	4.00 ± 0.260	4.10 ± 0.337	3.98 ± 0.208
Spleen (g)	0.71 ± 0.101	0.66 ± 0.114	0.64 ± 0.058	0.62 ± 0.084	0.63 ± 0.068
Per body weight (%)	0.19 ± 0.032	0.17 ± 0.028	0.17 ± 0.015	0.17 ± 0.020	0.17 ± 0.013
Kidneys (g)	2.15 ± 0.185	2.12 ± 0.178	2.15 ± 0.216	2.09 ± 0.232	1.98 ± 0.207
Per body weight (%)	0.56 ± 0.050	0.55 ± 0.044	0.56 ± 0.048	0.57 ± 0.045	0.53 ± 0.042
Thymus (g)	0.49 ± 0.063	0.50 ± 0.060	0.51 ± 0.081	0.44 ± 0.107	0.36 ± 0.101*
Per body weight (%)	0.13 ± 0.021	0.13 ± 0.015	0.13 ± 0.021	0.12 ± 0.022	0.10 ± 0.022*
Heart (g)	1.11 ± 0.105	1.16 ± 0.096	1.10 ± 0.081	1.15 ± 0.151	1.08 ± 0.119
Per body weight (%)	0.29 ± 0.022	0.30 ± 0.022	0.28 ± 0.021	0.31 ± 0.035	0.29 ± 0.024
Ovary (g)	0.15 ± 0.021	0.140 ± 0.017	0.15 ± 0.015	0.15 ± 0.033	0.13 ± 0.013
Per body weight (%)	0.04 ± 0.006	0.036 ± 0.004	0.04 ± 0.005	0.04 ± 0.010	0.03 ± 0.006

Values are presented as means ± SD (g).

***: Significantly different at $P < 0.05$ level when compared with the control group.**

Table 3 Serum biochemical values of pregnant rats treated with MWCNT during gestational days 6 -19

Parameters	Multi-wall carbon nanotubes (mg/kg/day)				
	0	8	40	200	1,000
No. of dams	11	9	10	12	11
Aspartate aminotransferase (IU/L)	73.9 ± 16.86	72.8 ± 12.69	66.9 ± 10.33	59.9 ± 19.62	61.8 ± 11.28
Alanine aminotransferase (IU/L)	38.2 ± 3.97	36.8 ± 4.20	37.6 ± 5.65	35.3 ± 6.73	35.4 ± 5.03
Total cholesterol (mg/dL)	105.1 ± 18.68	98.3 ± 18.21	94.6 ± 18.97	92.3 ± 18.87	95.0 ± 18.02
Triglyceride (mg/dL)	285.7 ± 109.48	326.7 ± 108.40	348.4 ± 107.86	308.8 ± 108.53	360.1 ± 109.73
Blood urea nitrogen (mg/dL)	20.1 ± 3.68	18.7 ± 2.66	19.6 ± 2.70	18.0 ± 3.26	17.4 ± 2.60
Alkaline phosphatase (IU/L)	923.6 ± 377.02	915.3 ± 368.65	903.7 ± 3.90	892.2 ± 374.21	921.7 ± 334.31
Creatinine (mg/dL)	0.4 ± 0.05	0.3 ± 0.04	0.4 ± 0.05	0.3 ± 0.05	0.3 ± 0.04
Glucose (mg/dL)	87.6 ± 24.59	89.3 ± 20.68	88.6 ± 23.20	94.6 ± 19.26	89.0 ± 25.61
Total bilirubin (mg/dL)	0.6 ± 0.08	0.5 ± 0.04	0.5 ± 0.31	0.6 ± 0.33	0.5 ± 0.07
Albumin (g/dL)	3.5 ± 0.38	3.1 ± 0.02	3.3 ± 0.12	3.2 ± 0.67	3.2 ± 0.02
Total protein (g/dL)	5.7 ± 0.48	5.3 ± 0.33	5.3 ± 0.26	5.3 ± 0.87	5.3 ± 0.34

Values are presented as means ± SD (g).

Table 4 Caesarean section data of pregnant rats treated with MWCNT during gestational day 6 -19

Parameters	Multi-wall carbon nanotubes (mg/kg/day)				
	0	8	40	200	1,000
No. of dams	11	9	10	12	11
No. of corpora lutea	14.7 ± 3.26	15.1 ± 2.76	14.6 ± 1.84	13.8 ± 2.12	14.8 ± 1.40
No. of implantation sites	11.6 ± 3.70	12.9 ± 2.47	11.9 ± 4.18	11.5 ± 3.63	14.0 ± 1.61
Pre-implantation loss (%) ^a	20.0 ± 20.49	14.2 ± 11.36	18.7 ± 25.95	19.1 ± 24.97	5.5 ± 7.02
Fetal deaths	0.5 ± 0.82	0.4 ± 0.88	0.5 ± 0.53	0.4 ± 0.67	0.5 ± 0.52
Post-implantation loss (%) ^b	3.1 ± 5.65	3.3 ± 6.67	3.6 ± 3.81	4.7 ± 6.30	3.2 ± 3.67
Litter size	11.2 ± 3.34	12.4 ± 2.51	11.4 ± 3.92	11.1 ± 3.70	13.5 ± 1.51
Sex ratio (male/female)	1.32	0.96	1.07	1.22	1.10
Fetal weight (g): Male	3.62 ± 0.194	3.63 ± 0.199	3.65 ± 0.219	3.42 ± 0.150	3.61 ± 0.181
Fetal weight (g): Female	3.39 ± 0.233	3.40 ± 0.236	3.50 ± 0.287	3.28 ± 0.150	3.41 ± 0.204
Placental weight (g)	0.53 ± 0.050	0.51 ± 0.059	0.58 ± 0.081	0.53 ± 0.057	0.51 ± 0.047

Values are presented as means ± SD (g).

a: Pre-implantation loss (%) = [(No. of corpora lutea - No. of implantation sites) / No. of corpora lutea] X 100

b: Post-implantation loss (%) = [(No. of implantation sites - No. of live embryos) / No. of implantation sites] X 100

Table 5 External and visceral alterations in fetuses from pregnant rats treated with MWCNT during gestational days 6 -19

Parameters	Multi-wall carbon nanotubes (mg/kg/day)			
	0	40	200	1,000
Litters examined	11	10	12	11
External examination				
-Fetuses examined	123	114	133	149
-Fetuses with malformations	0	0	0	0

-Litters affected	0	0	0	0
Visceral examination				
-Fetuses examined	59	57	66	72
-Fetuses with malformations	0	0	0	0
-Litters affected	0	0	0	0
-Fetuses with variations (%) ^a	5 (8.5)	7 (12.3)	5 (7.6)	6 (7.8)
-Litters affected (%) ^b	3 (27.3)	4 (40)	3 (25)	4 (36.4)
-Misshapen thymus	2	3	2	4
-Dilated ureter	3	4	3	2

a: A single fetus may be represented more than once in listing individual defects.

b: Includes litters with one or more affected fetuses.

Table 6 Skeletal alterations in fetuses from pregnant rats treated with MWCNT during gestational days 6 -19

Parameters	Multi-wall carbon nanotubes (mg/kg/day)			
	0	40	200	1,000
Fetuses examined	64	57	67	77
Litters examined	11	10	12	11
Fetuses with malformations ^a	0	0	0	0
Litters affected ^b	0	0	0	0
Fetuses with variation (%) ^a	12 (18.8)	13 (22.8)	15 (22.4)	16 (20.8)
Litters affected (%) ^b	7 (63.6)	5 (50)	6 (50)	6 (54.5)
Enlarged fontanel	4	1	0	1
Incomplete ossification of interparietal	1	1	0	7
Bipartite ossification of sternebrae	0	0	1	1
Misshapen sternebrae	3	4	2	0
Short supernumerary ribs	0	0	0	1
Dumbbell ossification of thoracic centrum	4	2	3	3
Bipartite ossification of thoracic centrum	1	2	1	0
Bipartite ossification of lumbar centrum	1	0	1	0
Supernumerary lumbar centrum	1	0	0	0
Incomplete ossification of pubis	1	3	0	5
Incomplete ossification of ischium	1	0	0	0

a: A single fetus may be represented more than once in listing individual defects.

b: Includes litters with one or more affected fetuses.

Applicant's summary and conclusion

Conclusions

MWCNTs (Hanhwa CM-95) were orally administered to pregnant rats from gestational day (GD) 6 through 19 at dose levels of 0, 8, 40, 200 and 1000 mg/kg bw/day. During the test period, clinical signs, mortality, body weights, food consumptions, serum biochemistry, oxidant-antioxidant status, gross findings, organ weights, and Caesarean section findings were examined. As a result, all dams survived to

the end of the study. A decrease in thymus weight was observed in the highest dose group. However, maternal body weight, food consumption, serum biochemical parameters, and oxidant-antioxidant balance in the kidneys were not affected by treatment with MWCNTs. No-treatment related differences in gestation index, fetal deaths, fetal and placental weights, or sex ratio were observed between the groups. Morphological examinations of the fetuses demonstrated no significant difference in the incidences of abnormalities between the groups. In conclusion, the results showed that the repeated oral dose of MWCNTs during pregnancy induced minimal maternal toxicity and no embryo-fetal developmental toxicity at 1000 mg/kg bw/day in rats. The NOAEL of MWCNTs is considered to be 200 mg/kg bw/day for dams and 1000 mg/kg bw/day for embryo-fetal development.

7.9 Specific investigations

7.10 Exposure related observations in humans

7.10.1 Health surveillance data

7.10.2 Epidemiological data

7.10.3 Direct observations: clinical cases, poisoning incidents and other

7.10.4 Sensitisation data (humans)

7.10.5 Exposure related observations in humans: other data

Endpoint study record: Hanwha CM-100 (1) Exposure related observations in humans: other data.001

Administrative Data

Purpose flag supporting study

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Han JH, Lee EJ, Lee JH, So KP, Lee YH, Bae GN, Lee SB, Ji JH, Cho MH, Yu IJ.	2008	Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility,	Inhalation Toxicology, 20: 741-749, (2008)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials

Test material identity

Identifier	Identity
other:	Hanwha CM 100

Method

Any other information on materials and methods incl. tables

Sampling sites

The iron particle catalyst with an MgO supporter was prepared using a combustion method in a high-temperature heating furnace in laboratory A, as described in Figure 1. The catalyst powder was then loaded onto a ceramic (alumina) boat and placed in a thermal chemical vapor deposition (CVD) chamber. Before allowing hydrocarbon gas to flow into the chamber, the catalyst was reduced at 600°C in ambient hydrogen gas. The resulting MWCNT powder was then recovered from the ceramic boat outside the chamber, and transferred to a processing room for spraying (laboratory B). However, before being sprayed, the powder also underwent several post treatments, such as dispersion and functionalization with several steps of sonication, which are typical procedures for CNT processes. Plus, the powder was transferred to a blending room (laboratory C) to formulate composites.

Personal and area sampling

Air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (35 mm diameter, 0.8µm nominal pore-size, and 2 in. cowl) obtained from Environmetrics, Inc (cat No. 20-31-0-1401, Charleston USA). The samples were collected in the breathing zone using SKC-117 battery operated sampling pumps at a flow rate of 1.5-2.0 l/min. The sampling holders were also changed during the sampling period to avoid overload. The samplings were performed during the normal work period from 09:30 to 16:00. The typical duration of the air sampling lasted from 240 to 360 min. Some samples were also collected when placing the sampler 3-4 meters away from the manufacturing devices.

Real-time aerosol monitoring

The SMPS that consisted of an electrostatic classifier (Model 3080, TSI) equipped with a long-differential mobility analyzer (LDMA, Model 3081, TSI) and ultrafine condensation particle counter (UCPC, Model 3025, TSI) was used to monitor the particle size distribution, ranging from 14 to 630 nm in the electrical mobility diameter in the laboratories. Meanwhile, the APS (Model 3321, TSI) was used to observe the particle size distribution, ranging from 0.5 to 20 µm in the aerodynamic diameter. In addition, a portable aethalometer (Model AE42-7-ER-MC, Magee Scientific) was utilized to measure the mass concentration of black carbon particles based on an optical absorption analysis. Here, the aethalometer sampled the total suspended particulate matter. The air in the laboratories was sampled at a flow rate of 0.3, 5, and 2 L/min for the SMPS, APS, and aethalometer, respectively. The SMPS scanned the particle sizes at a time resolution of 3 min (120 s for up-scan and 30 s for retrace), while the average time for the

APS and aethalometer was 150 s and 2 min, respectively. The real-time monitoring of aerosols was performed in relation to the metal blending process in laboratory C for approximately one and half hours before the implementation of the protective control measures.

Description of exposure control measures

After the initial MWCNT exposure measurements, a simple fan was installed in laboratories A and B, along with some simple cleaning and rearrangement. However, since laboratory C was found to be severely exposed to MWCNT powder, the composite blender was separated by a simple compartment into area 1, where the blender was located, and area 2, where the blender was excluded. In addition, since the chiller used to cool the furnace produced severe vibrations, it was relocated outside the building.

MWCNT counting and asbestos analysis

The air samples were analyzed according to the NIOSH analytical method 7402 (1994). The filter was coated with carbon and mounted onto carbon-coated nickel grids (Veco, Eerbeek, Holland) using chloroform vapor. The asbestos fibers were morphologically identified using a scanning transmission electron microscope (STEM, Hitachi 7100, Tokyo), while the MWCNT and asbestos types were determined by comparing the elemental composition of the asbestos fibers using an energy dispersive X-ray analyzer (EDX, KEVEX 7000Q, Foster City, CA). All tubes and fiber structure aspect ratios greater than 3:1 were counted in more than 50 grid openings at random.

Results and discussions

Any other information on results incl. tables

Exposure to MWCNTs before and after control measures.

The total particle concentrations in the CNT laboratory were below the current ACGIH TLVs for carbon black (3.5 mg/m³) and PNOs (particle not otherwise specified, 3 mg/m³). However, the STEM morphological examination indicated potential exposure to both MWCNTs and other particles (Figure 1). The main particle components were Fe and carbon, while the MWCNTs contained no Fe or Ni at all (Figure 2), yet metals such as Fe and Ni are considered to be closely related to the use of metal catalysts during CNT manufacturing. The potential exposure to MWCNTs was greatly reduced in terms of the total particles and number of MWCNTs after enclosing and ventilating the furnace, and placing the chiller outside (Tables 1 and 2, Lab C). None of the personal or area samples measured inside the furnace enclosure in Lab C showed any measurable particle mass concentrations. Plus, after enclosing the furnace in Lab C, the number of MWCNTs was also greatly reduced in both the personal and area samples. Meanwhile, the installation of a simple fan in Labs A and B did not have much impact on reducing the potential exposure to MWCNTs (Table 2). The diameter and length of the tubes in the personal and area samples from Lab C were 52-56 nm and 1473-1760 nm, respectively. Plus, the STEM analysis revealed various MWCNT shapes, ranging from an individual tube structure to clumped tube structures (Figure 1), along with a strong tendency to bundle together in “ropes” due to van der Waals forces analogous to forces that bind sheets of graphite (Thess et al., 1996).

Detection of asbestos fiber-like structures in laboratory

The samples taken from CNT Labs A and C were found to contain asbestos fiber-like structures. The fibers were mostly chrysotile fibers, as determined from the STEM-EDX analysis, the fiber concentrations ranged from 0.01 to 1.56 fibers/cc (Table 3), and the fiber lengths were generally shorter than 5µm (Table 4). No asbestos fiber-like structures were detected after installing the protective control measures, except for a very small amount inside the furnace enclosure.

Real-time monitoring of particles in laboratory C

The changes in the particle number concentration measured by the SMPS and APS, and black carbon mass concentration measured by the aethalometer during the metal blending in Lab C are shown in Figure 3, plus a time course of events is presented in Table 5. The number of particles within a size range

of 14 to 630 nm reached over 30,000 particles/cm³ when operating the rotary-type oil vacuum pump, whereas the particle number before operating the vacuum pump was only around 10,000 particles/cm³. When operating the rotary-type oil vacuum pump, a mist also filled the room, along with a combustion smell. According to statements from the lab workers, proper extraction of the oil mist was often inadequate due to ineffective operation of the ventilation duct system. Nonetheless, the high number of particles produced rapidly at that time returned to the basal level within 20 minutes (Figure 3A). As shown in Figure 5B, the particle size distribution of the oil mist was unimodal, with a mode diameter of about 300 nm. From TEM images of short-term air sampling, it was confirmed that MWCNTs were not released during the oil-mist period. Plus, no difference was found in the particle size distribution, except for some particles larger than 300 nm, between the indoor background condition and when the blending equipment was open.

When the blending equipment was opened the second time, an increase in the particle number concentration was easily detected from the APS data, yet not from the SMPS data, as shown in Figure 5A. As such, the relatively high background concentration of small particles detected by the SMPS may have prohibited the detection of MWCNTs, whereas the relatively low background concentration of large particles detected by the APS did not disturb the detection of MWCNTs, as shown in Figures 3A and 3C. Although the size-segregated number concentrations when opening the blending equipment were significantly higher than those for the background condition, as shown in Figure 5C, the size distribution data did not confirm the release of MWCNTs. To identify the MWCNTs signature, the particle size distributions were normalized, as shown in Figure 3D, and a significant difference was observed for particle diameters of 2 to 4 µm between the normalized particle size distributions for the indoor background condition and those when opening the blending equipment, indicating the release of MWCNTs. This size response of MWCNTs was similar to that previously reported for SWCNT aerosols generated using a vortex shaker fluidized bed in a controlled laboratory experiment (Maynard et al., 2004).

The black carbon concentration increased up to 200 µg/m³ when the blending equipment was opened the second time, as shown in Figure 5A, which may have indicated the release of MWCNTs. The black carbon was not monitored during the mist period, as the filter tape of the aethalometer was automatically advanced. In addition to the chemical composition, the structural properties of particles are helpful to anticipate the response of a specific particle size caused by CNTs. These properties can be ascertained based on a mass measurement of discretely singly-charged airborne particles using an aerosol particle mass analyzer (McMurry et al., 2002; Maynard et al., 2007).

Overall remarks, attachments

Attached full study report

Attached full study report
Human exposure _Korea_.pdf / 2.72 MB (application/pdf): Appendix 1

Applicant's summary and conclusion

Conclusions

The gravimetric concentrations of total dust before any control measures ranged from 0.21 to 0.43 mg/m³, then decreased to a non-detectable level after implementing the control measures. The number of MWCNTs in the samples obtained from the MWCNT blending laboratory ranged from 172.9-193.6 MWCNTs/cc before the control measures, and decreased to 0.018-0.05 MWCNTs/cc after the protective improvements. The real-time monitoring of aerosol particles provided a signature of the MWCNTs released from the blending equipment in laboratory C. In particular, the number size response of an aerodynamic particle sizer with a relatively high concentration in the range of 2 to 3 µm in aerodynamic diameter revealed the evidence of MWCNT exposure. The black carbon mass concentration also increased significantly during the MWCNT release process. Therefore, the present study suggests that the

conventional industrial hygiene measures can significantly reduce exposure to airborne MWCNTs and other particulate materials in a nano research facility.

Endpoint study record: Hanwha CM 100 (2) Exposure related observations in humans: other data.001

Administrative Data

Purpose flag supporting study

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Lee JH, Lee SB, Bae GN, Jeon KS, Yoon JU, Ji JH, Sung JH, Lee BG, Lee JH, Yang JS, Kim HY, Kang CS, Yu IJ.	2010	Exposure assessment of carbon nanotube manufacturing workplaces,	Inhalation Toxicology, 22(5):369-81, (2010)					

Materials and methods

Method

Any other information on materials and methods incl. tables

Sampling sites

The current study measured the nanoparticle concentrations inside various plants during July-September, 2008. The seven plants tested were mostly involved in MWCNT manufacturing and handling. The information related to each plant is shown in Table 1.

Personal and area sampling

The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8µm nominal pore-size, and 2 in. cowl, open-face) obtained from Pall Corp (P/N 64678, Michigan USA). The filter samples were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5-2.0 L/min. The sampling holders were also changed during the sampling period to avoid overload. The samplings were performed during the normal work period from 09:30 to 16:00. The typical duration of the air sampling lasted from 183 to 409 min. Some samples were also collected when placing the sampler 1-4 meters away from the manufacturing devices.

Real-time aerosol monitoring

An SMPS combining a differential mobility analyzer (Short type-DMA, 4220, HCT Co., Ltd, Korea; range 5 ? 300 nm) and condensation particle counter (CPC, 4312, HCT Co., Ltd, 0 ? 10⁸ particles/cm³ detection range) was used to monitor the particle size distribution with an electrical

mobility diameter ranging from 14 to 685 nm. Meanwhile, a dust monitor (Model 1.109, Grimm) was used to observe the particle size distribution with a diameter ranging from 0.25 to 32 μm . The total number concentration of particles larger than 3 nm was monitored by using an ultrafine particle counter (UCPC, 3025, TSI, 0-100,000 particles/ cm^3 detection range). In addition, a portable aethalometer (Model AE42-7-ER-MC, Magee Scientific) was utilized to measure the mass concentration of black carbon particles based on an optical absorption analysis. Here, the aethalometer sampled the total suspended particulate matter. The air in the laboratories was sampled at a flow rate of 0.3, 1.2, and 2 L/min for the SMPS, dust monitor, and aethalometer, respectively. The SMPS scanned the particle sizes at a time resolution of 3 min (120 s for up-scan and 30 s for retrace), while the average times for the dust monitor and aethalometer were 6 sec~5 min and 2 min, respectively.

. MWCNT counting

The air samples were analyzed according to NIOSH analytical method 7402 (1994) and Han et al. (2008). The filter was coated with carbon and mounted onto carbon-coated copper grids (Veco, Eerbeek, Holland) using acetone vapor. Meanwhile, the CNTs were morphologically identified using a scanning transmission electron microscope (STEM, Hitachi 7100, Tokyo) and determined by comparing the elemental composition of the carbon nanotube using an energy dispersive X-ray analyzer (EDX, KEVEX 7000Q, Foster City, CA) (Han et al., 2008). All tubes and fiber structure aspect ratios greater than 3:1 were counted in more than 50 grid openings at random.

Results and discussions

Any other information on results incl. tables

Refer to Attached Report

Overall remarks, attachments

Attached full study report

Attached full study report
Human exposure _Korea_.pdf / 2.72 MB (application/pdf): Appendix 1

7.11 Toxic effects on livestock and pets

7.12 Additional toxicological information

Endpoint study record: Baytubes: Additional toxicological information.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	4 (not assignable)
Rationale for reliability incl. deficiencies	poor documentation regarding the test method and results

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Bayer MaterialScience	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Materials and methods**Type of information**

in vitro study

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Method**Any other information on materials and methods incl. tables**

Permeability assay with Caco-2 cells

Results and discussions**Any other information on results incl. tables**

negative

Endpoint study record: Baytubes: Additional toxicological information.002**Administrative Data****Purpose flag**

supporting study

Study result type

experimental result

Reliability

4 (not assignable)

Rationale for reliability incl. deficiencies poor documentation regarding the test method and results

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Bayer MaterialScience	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Materials and methods**Type of information**

in vitro study

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Method**Any other information on materials and methods incl. tables**

Acute toxicity tests with MWCNTs were performed using human lung cells (A549) to determine endpoints like proliferation and vitality of cells, inflammation factors and immune response.

Results and discussions**Any other information on results incl. tables**

No acute toxicity was detected.

Endpoint study record: Baytubes: Additional toxicological information.003**Administrative Data****Purpose flag**

supporting study

Study result type

experimental result

Reliability

4 (not assignable)

Rationale for reliability incl. deficiencies poor documentation regarding the test method and results.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Bayer MaterialScience	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Method**Any other information on materials and methods incl. tables**

A 3D skin model was developed to investigate a potential penetration of MWCNTs through the skin and to monitor an irritative effect upon chronic exposure.(GER/Tracer project)

Results and discussions**Any other information on results incl. tables**

No penetration of MWCNTs was found.

Endpoint study record: Graphistrength C100: Additional toxicological information.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well-documented publication

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ronzani C, Spiegelhalter C, Vonesch JL, Lebeau L, Pons F.	2012	Lung deposition and toxicological response evoked by multi-walled carbon nanotubes dispersed in a synthetic lung surfactant in the mouse	Arch. toxicol., 86, 137-149 (2012)					

Data access

data published

Materials and methods**Type of information**

in vivo study

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

GLP compliance

no data

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Method**Any other information on materials and methods incl. tables**

Mice were exposed to surfactant dispersed MWCNT according to single or repeated protocols. In the single exposure protocol, mice received one administration of MWCNT and were used 24 h later. In the repeated exposure protocol, mice were given MWCNT on days 0, 7 and 14 and were used on day 21. Taking into account this exposure protocols and CNT dose of 10-20µg/mouse as relevant to occupational human exposure, doses of MWCNT used in the present study were set to 1.5, 6.25 and 25µg. These doses were administered into the lung of mice by intranasal instillation

of 25 μ L of a 1 mg/4mL dispersion (6.25 μ g), a 4-time dilution of this dispersion (1.5 μ g) or a 4 mg/4 mL preparation (25 μ g). Control animals received same amount of surfactant or saline. In evaluation for lung inflammatory response, cytokine assay in BALF, collagen assay in lung and histological observation of lungs were done.

Results and discussions

Any other information on results incl. tables

Histology on lung tissue section demonstrated that a surfactant-dispersed MWCNT distributed all throughout the mouse airways upon single and repeated administrations and were observed in alveolar macrophages and epithelial cells, and in infiltrated neutrophils. Mice that received a single administration of MWCNT (6.25 μ g/mouse) showed neutrophil infiltrate and greater concentrations of TNF- α , keratinocyte-derived chemokine (KC) and interleukin-17 in BALF when compared to controls. After repeated MWCNT administrations (25 μ g/mouse), increases in macrophage number, KC and TGF- β 1 levels in BALF, and collagen deposition and mucus hyperplasia in lung tissue were observed.

Overall remarks, attachments

Illustration (picture/graph)

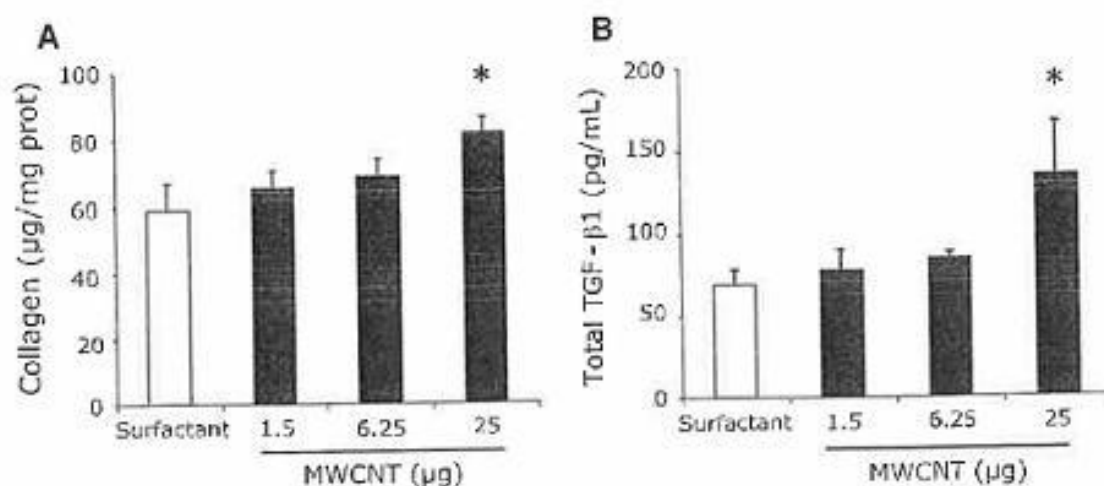


Fig Airway remodeling induced by surfactant-dispersed MWCNT

Applicant's summary and conclusion

Conclusions

Mice were exposed to surfactant-dispersed MWCNT by single or repeated intranasal instillation. Inflammatory responses in the lungs were evaluated by cytokine assay in BALF, collagen in lung and histological observation of the lungs. Histological lung tissue sections demonstrated that a surfactant-dispersed MWCNT distributed all throughout of the mouse airways upon single and repeated administration. In single exposure, mice exposed to 6.25 μ g MWCNT revealed neutrophil infiltration and greater concentrations of cytokines in BALF. After repeated administration of MWCNT, increases in

macrophage number, cytokine levels in BALF, and collagen deposition and mucus hyperplasia in lung tissue were observed at a dose of 25 ug.

Endpoint study record: Graphistrength C100: Additional toxicological information.002

Administrative Data

Purpose flag supporting study
Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability incl. deficiencies Acceptable, well-documented publication

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Arkema	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						
publication	Tabet L, Bussy C, Amara N, Setyan A, Grodet A, Rossi MJ, Pairon JC, Boczkowski J, Lanone S.	2009	Adverse effects of industrial multiwalled carbon nanotubes on human pulmonary cells	J. Toxicol. Environ. Health, 72(2), 60-73 (2009)					

Data access

data published

Materials and methods

Type of information

in vitro study

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

GLP compliance

no

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Method

Any other information on materials and methods incl. tables

In order to evaluate the potential adverse effects of MWCNT in A549 Human alveolar epithelial cells and MT5A mesothelial cells, a comprehensive approach was used, aimed to evaluate both cytotoxic effects and the underlying mechanisms (apoptosis, proliferation, oxidative stress, internalization). MWCNT was dispersed in dipalmitoyl lecithin (DPL), a component of pulmonary surfactant.

Effects of MWCNT were also compared to those of 2 asbestos fibers (chrysotile and crocidolite) and carbon black (CB) nanoparticles, not only in A549 cells, but also on methothelial cell (MeT5A human cell line), used as asbestos-sensitive cell line.

Human alveolar epithelial cells (A549 cell line, ATCC, France) and mesothelial cells (MeT5A cell line, ATCC, France) were cultured, seeded in 96-well plates at 60 000 cells/ml and grown to confluence (48 to 72 hr later). Cells were exposed for 6, 24, 48 or 72 hr to serum-free medium, 0.1 to 100 µg/ml (0.02 - 20 µg/cm²) of MWCNT, or 100 µg/ml (20 µg/cm²) asbestos fibers or CB nanoparticles. The final concentration of DPL, PBS, or EtOH in the culture medium was 1% for each condition of stimulation, a concentration that did not elicit any cell toxicity (data not shown). As MeT5A cells were used only to verify the main results obtained with A549 cells, these cells were exposed only to DPL-suspended MWCNT at different concentrations (0.1 to 100 µg/ml).

-Assessment of cell viability: Two methods were used to evaluate changes in cell viability. MTT, and Neutral Red assays.

-Assessment of cell number: Cell number was assessed by quantifying DNA content with fluorescent dye bisbenzamide (Hoechst 33258)

-Assessment of apoptosis: Apoptosis was examined by DAPT staining coupled to fluorescence microscopy analysis in the same experimental setting that used for assessment of cell number.

-Assessment of cell proliferation: The effects of nanomaterials on cell proliferation were determined by bromodeoxyuridine (BrdU) cell proliferation ELISA.

-Assessment of internalization: Cells exposed for 48 hr to different nanomaterials at 100 µg/ml were analyzed by TEM.

-Markers of oxidative stress: Oxidative stress was evaluated by analyzing mRNA expression of the anti- and pro-oxidant systems HO-1, SOD2, GPx and NOX4 respectively by quantitative real-time RT-PCR by use of the PCR ABI 7700 apparatus (Applied Biosystems), after exposure of cells for 6 or 24 hr up to 100µg/ml nanoparticles.

Results and discussions

Any other information on results incl. tables

For the MTT assay, MWCNT effects on A549 cell viability were similar for the 3 dispersion media.

Compared with respective controls (cells cultured in media containing the respective suspension agent without particles), MTT values decreased significantly with 10 µg/ml to 100 µg/ml incubation, reaching 60% of control values for 100 µg/ml incubation at 48 hr post-exposure (for 10 and 100 µg/ml). Similar results were obtained after exposure of MeT5A cells to DPL-suspended MWCNT. In both cell types, incubation with 100

µg/ml chrysotile or crocidolite elicited similar decreases in MTT, which were not different from that induced by 100 µg/ml MWCNT after 24 hr.

Neither MWCNT cellular internalization nor oxidative stress was observed. In contrast, asbestos fibers penetrated into the cells, decreased metabolic activity but not cell membrane permeability, and increased apoptosis, without decreasing cell number. Carbon black was internalized without any adverse effects. In conclusion, this study demonstrates that Graphistrength C100 exert adverse effects without being internalized by human epithelial and mesothelial pulmonary cell lines.

Applicant's summary and conclusion

Conclusions

The aim of this study was to evaluate adverse effects of multi-walled carbon nanotubes (MWCNT) produced for industrial purposes, on the human epithelial cell line A549. MWCNT were dispersed in dipalmitoyl lecithin (DPL), a component of pulmonary surfactant, and the effects of dispersion in DPL were compared to those in 2 other media: ethanol (EtOH) and phosphate buffer saline (PBS). Effects of MWCNT were also compared to those of 2 asbestos fibers (chrysotile and crocidolite) and carbon black (CB) nanoparticles. Whatever the dispersion media, incubation with 100 µg/ml MWCNT induced a similar decrease in metabolic activity without changing cell membrane permeability or apoptosis. Neither MWCNT cellular internalization nor oxidative stress were observed. In contrast, asbestos fibers penetrated into the cells, decreased metabolic activity but not cell membrane permeability and increased apoptosis, without decreasing cell number. CB was internalized without any adverse effects. In conclusion, this study demonstrates that MWCNT produced for industrial purposes exert adverse effects without being internalized by human epithelial and mesothelial pulmonary cell lines.

Endpoint study record: Mitsui MWNT-7: Additional toxicological information.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well-documented publication

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Asakura M, Sasaki T, Sugiyama T, Takaya M, Koda S, Nagano K, Arito H, Fukushima S.	2010	Genotoxicity and Cytotoxicity of Multi-wall Carbon Nanotubes in Cultured Chinese Hamster Lung Cells in Comparison with Chrysotile A Fiber	J. Occup Health, 52: 155-166, (2010)					

Data access

data published

Materials and methods**Type of information**

in vitro study

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

GLP compliance

yes

Test materials**Test material identity**

Identifier	Identity
other:	Mitsui MWNT-7

Details on test materialMean and SD of the length: $5.0 \pm 4.5 \mu\text{m}$ Mean and SD of the width: $88 \pm 5 \text{ nm}$ **Method****Any other information on materials and methods incl. tables**

The MWCNT was suspended in 1) direct suspension in Eagle's MEM supplemented with 10% heat-inactivated CS used as culture medium, 2) suspension in a mixture of DMSO and the culture medium with a final concentration of 0.5% (5.5 mg/ml), 3) suspension in an aqueous solution containing Tween 80 followed by addition of the ultrasonicated suspension in the culture medium with the final concentration of 0.1 mg/ml, 4) suspension in CMC solution followed by addition of the ultrasonicated suspension into the culture medium with the final concentration of 1 mg/ml. The peaks of hydrodynamic diameter distributions measured by DLS shifted from larger to smaller in the order of Tween 80, CMC, culture medium, and

DMSO/culture medium,.

Cytotoxicity was assayed by colony forming assay and LDH assay.1) Colony forming assay: One hundred CHL/IU cells (20 cells/ml x 5 ml) of single cell suspension were seeded in a 60-mm plastic culture dish, incubated in a culture medium for 24 h, and then replaced with the test substance suspended in culture medium. The cells were exposed to MWCNT at 12.5 to 400 ug/mL or chrysotile at 0.25 to 50 ug/mL for 7 days. Then, the cells were fixed with ethanol for 5 min and stained with 0.1% crystalviolet for 20 min, and colonies were counted under a microscope. The percentage of surviving cells (viability) was calculated by dividing the number of colonies formed during MWCNT treatment by the number of colonies in the control medium and multiplying by 100.2) LDH assay: One hundredthousand cells (20,000 cells/ml x 5 ml) were seeded in a 60-mm plastic culture dish, incubated in a culture medium for 48 h, and then replaced with the test substance suspended in culture medium. The cells were exposed to MWCNT at 12.5 to 400 ug/mL or chrysotile at 10 ug/mL for 24 h. The medium containing the test substance was then centrifuged at 13,000 rpm for 5 minutes. The LDH concentration in the supernatant was measured by a clinical analyzer. The remaining cells in the dish were transferred to a 0.1% triton X-100 solution to extract LDH inside the cells, and then centrifuged at 13,000 rpm for 5 minutes. The LDH in the supernatant was measured similarly. The LDH release rate was calculated by dividing the concentration of LDH in the test substance suspended in the culture medium by the total concentration of LDH inside and outside the cells and multiplying by 100.

Results and discussions

Any other information on results incl. tables

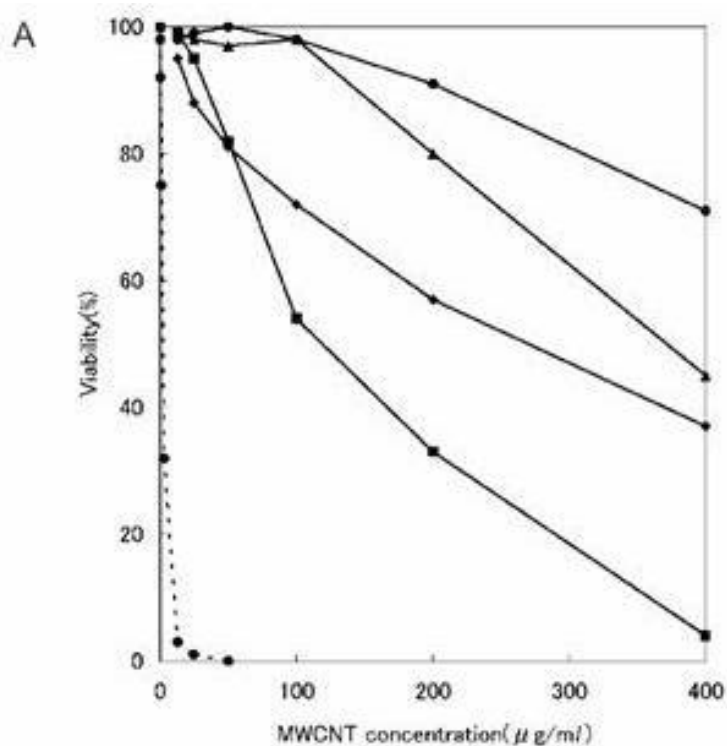
The in vitro cytotoxicity of MWCNT depended on the solvent used for suspension of MWCNT and ultrasonication duration of the MWCNT suspension. A combination of DMSO/culture and 3 -minute ultrasonication resulted in a well-dispersed medium with dispersion and isolation of agglomerated MWCNT by ultrasonication which manifested the highest cytotoxicity.

Both colony formation and LDH assays exhibited clear but different dose-response curves for cytotoxicity. It was also found that the LDH release rate of chrysotile at 10 ug/ml was greater than that expected from the dose-response curve of MWCNT, indicating that chrysotile damaged the cells more severely than MWCNT.

Overall remarks, attachments

Attached full study report

Illustration (picture/graph)



Effect of solvent used for MWCNT suspension on its cytotoxicity with CHL/IU cells. —●— culture medium, —■— DMSO/culture medium, —▲— Tween 80, —◆— 1%CMC, -●-chrysotile (water).

Applicant's summary and conclusion

Conclusions

Cytotoxicity of MWCNT and chrysotile was evaluated using CHL/IU cells. Both colony formation and LDH assays exhibited clear but different dose-response curves for cytotoxicity. Cytotoxicity was more potent for chrysotile than MWCNT.

8. ANALYTICAL METHODS

9. RESIDUES IN FOOD AND FEEDINGSTUFFS

10. EFFECTIVENESS AGAINST TARGET ORGANISMS

11. GUIDANCE ON SAFE USE

12. LITERATURE SEARCH

13. ASSESSMENT REPORTS

14. INFORMATION REQUIREMENTS

APPENDIX

Appendix 1: Human exposure _Korea_.pdf / 2.72 MB (application/pdf)

Human Exposure Multi-walled Carbon Nanotubes (MWCNTs)

Sponsor: Republic of Korea

Experience with human exposure

Test substances: MWCNTs at various manufacturing facilities

Sampling sites

The iron particle catalyst with an MgO supporter was prepared using a combustion method in a high-temperature heating furnace in laboratory A, as described in Figure 1. The catalyst powder was then loaded onto a ceramic (alumina) boat and placed in a thermal chemical vapor deposition (CVD) chamber. Before allowing hydrocarbon gas to flow into the chamber, the catalyst was reduced at 600°C in ambient hydrogen gas. The resulting MWCNT powder was then recovered from the ceramic boat outside the chamber, and transferred to a processing room for spraying (laboratory B). However, before being sprayed, the powder also underwent several post treatments, such as dispersion and functionalization with several steps of sonication, which are typical procedures for CNT processes. Plus, the powder was transferred to a blending room (laboratory C) to formulate composites.

Personal and area sampling

Air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (35 mm diameter, 0.8µm nominal pore-size, and 2 in. cowl) obtained from Environmetrics, Inc (cat No. 20-31-0-1401, Charleston USA). The samples were collected in the breathing zone using SKC- 117 battery operated sampling pumps at a flow rate of 1.5-2.0 l/min. The sampling holders were also changed during the sampling period to avoid overload. The samplings were performed during the normal work period from 09:30 to 16:00. The typical duration of the air sampling lasted from 240 to 360 min. Some samples were also collected when placing the sampler 3-4 meters away from the manufacturing devices.

Real-time aerosol monitoring

The SMPS that consisted of an electrostatic classifier (Model 3080, TSI) equipped with a long-differential mobility analyzer (LDMA, Model 3081, TSI) and ultrafine condensation particle counter (UCPC, Model 3025, TSI) was used to monitor the particle size distribution, ranging from 14 to 630 nm in the electrical mobility diameter in the laboratories. Meanwhile, the APS (Model 3321, TSI) was used to observe the particle size distribution, ranging from 0.5 to 20 µm in the aerodynamic diameter. In addition, a portable aethalometer (Model AE42-7-ER-MC, Magee Scientific) was utilized to measure the mass concentration of black carbon particles based on an optical absorption analysis. Here, the aethalometer sampled the total suspended particulate matter. The air in the laboratories was sampled at a flow rate of 0.3, 5, and 2 L/min for the SMPS, APS, and aethalometer, respectively. The SMPS scanned the particle sizes at a time resolution of 3 min (120 s for up-scan and 30 s for retrace), while the average time for the APS and aethalometer was 150 s and 2 min, respectively. The real-time monitoring of aerosols was performed in relation to the metal blending process in laboratory C for approximately one and half hours before the implementation of the protective control measures.

Description of exposure control measures

After the initial MWCNT exposure measurements, a simple fan was installed in laboratories A and B, along with some simple cleaning and rearrangement. However, since laboratory C was found to be severely exposed to MWCNT powder, the composite blender was separated by a simple compartment into area 1, where the blender was located, and area 2, where the blender was excluded. In addition, since the chiller used to cool the furnace produced severe vibrations, it was relocated outside the building.

MWCNT counting and asbestos analysis

The air samples were analyzed according to the NIOSH analytical method 7402 (1994). The

filter was coated with carbon and mounted onto carbon-coated nickel grids (Veco, Eerbeek, Holland) using chloroform vapor. The asbestos fibers were morphologically identified using a scanning transmission electron microscope (STEM, Hitachi 7100, Tokyo), while the MWCNT and asbestos types were determined by comparing the elemental composition of the asbestos fibers using an energy dispersive X-ray analyzer (EDX, KEVEX 7000Q, Foster City, CA). All tubes and fiber structure aspect ratios greater than 3:1 were counted in more than 50 grid openings at random.

Results

Exposure to MWCNTs before and after control measures.

The total particle concentrations in the CNT laboratory were below the current ACGIH TLVs for carbon black (3.5 mg/m^3) and PNOSs (particle not otherwise specified, 3 mg/m^3). However, the STEM morphological examination indicated potential exposure to both MWCNTs and other particles (Figure 1). The main particle components were Fe and carbon, while the MWCNTs contained no Fe or Ni at all (Figure 2), yet metals such as Fe and Ni are considered to be closely related to the use of metal catalysts during CNT manufacturing. The potential exposure to MWCNTs was greatly reduced in terms of the total particles and number of MWCNTs after enclosing and ventilating the furnace, and placing the chiller outside (Tables 1 and 2, Lab C). None of the personal or area samples measured inside the furnace enclosure in Lab C showed any measurable particle mass concentrations. Plus, after enclosing the furnace in Lab C, the number of MWCNTs was also greatly reduced in both the personal and area samples. Meanwhile, the installation of a simple fan in Labs A and B did not have much impact on reducing the potential exposure to MWCNTs (Table 2). The diameter and length of the tubes in the personal and area samples from Lab C were 52-56 nm and 1473-1760 nm, respectively. Plus, the STEM analysis revealed various MWCNT shapes, ranging from an individual tube structure to clumped tube structures (Figure 1), along with a strong tendency to bundle together in “ropes” due to van der Waals forces analogous to forces that bind sheets of graphite (Thess et al., 1996).

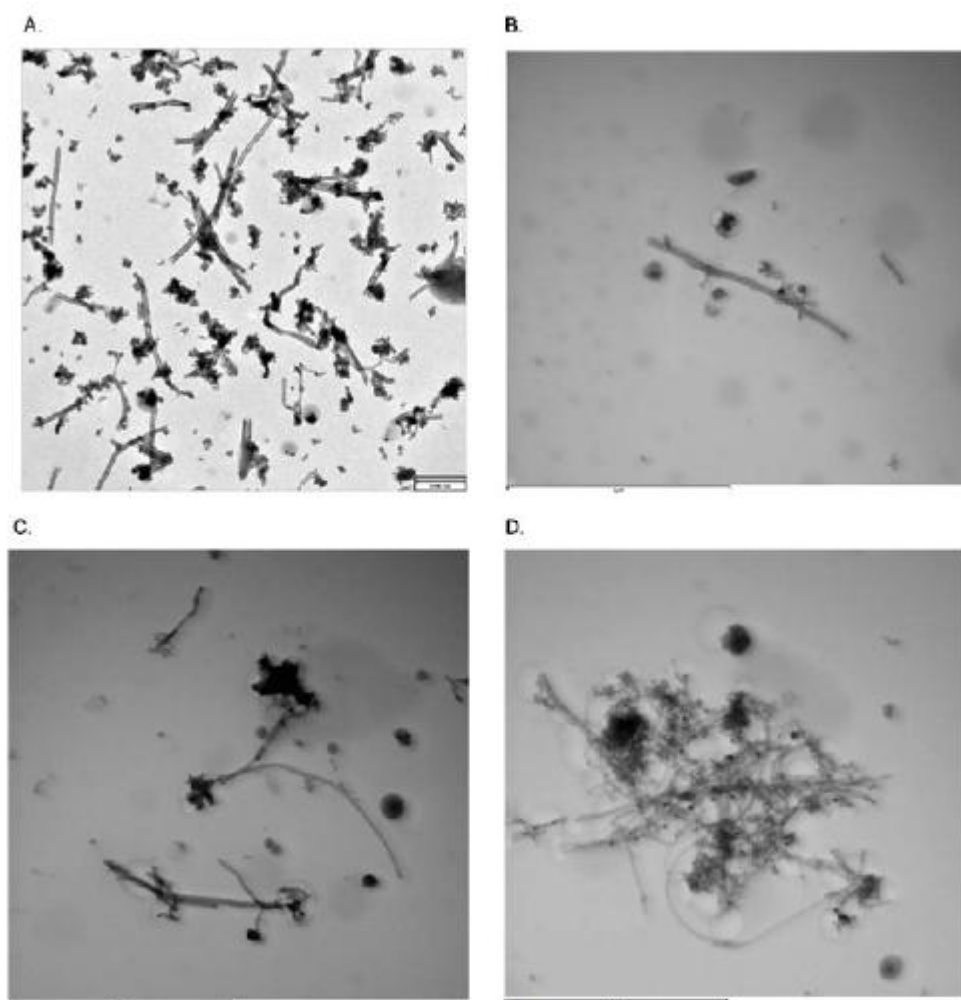


Figure 1. Shapes of MWCNTs. The bars in the B-C indicate 4 μm . A. MWCNTs sampled after opening of the furnace. B. Individual tube structure, C. Multiple tube structure D. Clumped tube structure

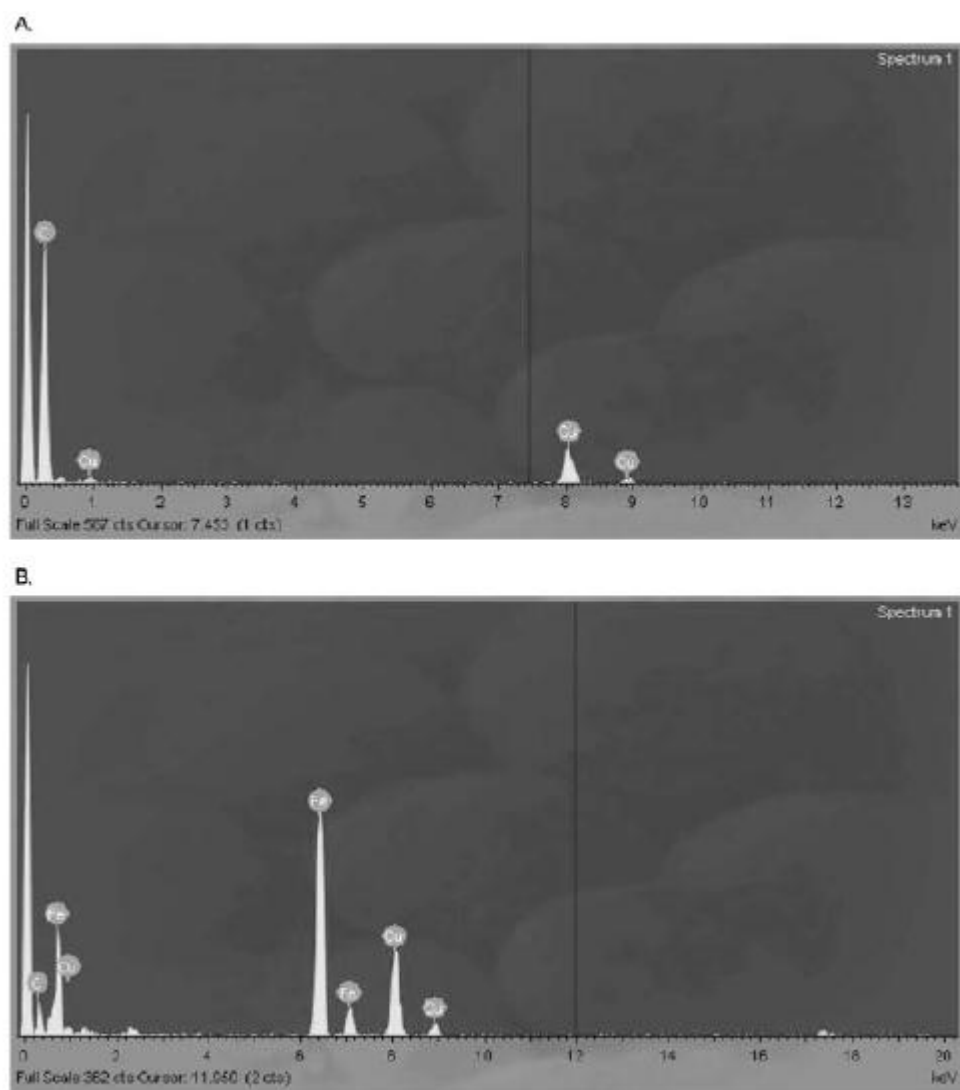


Figure 2.. STEM-EDX analysis of MWCNTs and particles using copper grids. A. CNT. Carbon was a major component. B. Particles. Fe and carbon were major components.

TABLE 1
Total Particle Concentration in CNT Laboratory Before and
After Installation of Engineering Equipment (mg/m³)

Lab	Sample	Operation	Before	After
A	Personal	Thermal CVD	ND	ND
	Area	Thermal CVD	ND	0.0388
	Personal	Al/CNT ball milling	ND	NM
B	Area	Weighing	0.1133	ND
	Area	Weighing/ spraying	0.0366	NM
	Personal	CNT solution spraying	0.1930	0.0309
C	Personal	Blending	0.3317 (open blender)	ND (inside encapsulation)
	Area	Blending	0.4345 (open blender)	ND (inside encapsulation)
	Area	Blending	0.2090	ND

Note. ND, not detected; NM, not measured.

TABLE 2
Number of CNTs in CNT Laboratory Before and After
Installation of Engineering Measures (Tubes/cm³)

Lab	Sample	Before	After
A	Personal	ND	0.08
	Area	ND	ND
	Personal	ND	0.018
B	Area 1	ND	1.997
	Area 2	ND	NM
	Personal	ND	0.008
C	Personal	193.6 (open)	0.018 (inside encapsulation)
	Area 1	172.9 (open)	0.05 (inside encapsulation)
	Area 2	NM	ND

Note. ND, not detected; NM, not measured.

Detection of asbestos fiber-like structures in laboratory

The samples taken from CNT Labs A and C were found to contain asbestos fiber-like structures. The fibers were mostly chrysotile fibers, as determined from the STEM-EDX analysis, the fiber concentrations ranged from 0.01 to 1.56 fibers/cc (Table 3), and the fiber lengths were generally shorter than 5 μm (Table 4). No asbestos fiber-like structures were detected after installing the protective control measures, except for a very small amount inside the furnace enclosure.

TABLE 3
Number of Asbestos Fibers in CNT Laboratory Before and
After Installation of Engineering Measures (Fibers/cm³)

Lab	Sample	Before	After
A	Personal	1.30	ND
	Area	0.01	ND
	Personal	ND	ND
B	Area 1	ND	ND
	Area 2	ND	ND
	Personal	ND	ND
C	Personal	ND (open)	ND
	Area 1	0.56 (open)	0.027 (inside encapsulation)
	Area 2	NM	ND

Note. ND, not detected; NM, not measured.

TABLE 4
MWCNT Diameter and Length Sampled From Laboratory C

Sample	Diameter (nm)	Length (nm)
Personal	52.3 ± 18.5	1472.8 ± 980.5
Area 2	56.0 ± 23.5	1760.2 ± 1198.3

Real-time monitoring of particles in laboratory C

The changes in the particle number concentration measured by the SMPS and APS, and black carbon mass concentration measured by the aethalometer during the metal blending in Lab C are shown in Figure 3, plus a time course of events is presented in Table 5. The number of particles within a size range of 14 to 630 nm reached over 30,000 particles/cm³ when operating the rotary-type oil vacuum pump, whereas the particle number before operating the vacuum pump was only around 10,000 particles/cm³. When operating the rotary-type oil vacuum pump, a mist also filled the room, along with a combustion smell. According to statements from the lab workers, proper extraction of the oil mist was often inadequate due to ineffective operation of the ventilation duct system. Nonetheless, the high number of particles produced rapidly at that time returned to the basal level within 20 minutes (Figure 3A). As shown in Figure 5B, the particle size distribution of the oil mist was unimodal, with a mode diameter of about 300 nm. From TEM images of short-term air sampling, it was confirmed that MWCNTs were not released during the oil-mist period. Plus, no difference was found in the particle size distribution, except for some particles larger than 300 nm, between the indoor background condition and when the blending equipment was open.

When the blending equipment was opened the second time, an increase in the particle number concentration was easily detected from the APS data, yet not from the SMPS data, as shown in Figure 5A. As such, the relatively high background concentration of small particles detected by the SMPS may have prohibited the detection of MWCNTs, whereas the relatively low background concentration of large particles detected by the APS did not disturb the detection of MWCNTs, as shown in Figures 3A and 3C. Although the size-segregated number concentrations when opening the blending equipment were significantly higher than those for the background condition, as shown in Figure 5C, the size distribution data did not confirm the release of MWCNTs. To identify the MWCNTs signature, the particle size distributions were normalized, as shown in Figure 3D, and a significant difference was observed for particle diameters of 2 to 4 µm between the normalized particle size distributions for the indoor background condition and those when opening the blending equipment,

indicating the release of MWCNTs. This size response of MWCNTs was similar to that previously reported for SWCNT aerosols generated using a vortex shaker fluidized bed in a controlled laboratory experiment (Maynard et al., 2004).

The black carbon concentration increased up to $200 \mu\text{g}/\text{m}^3$ when the blending equipment was opened the second time, as shown in Figure 5A, which may have indicated the release of MWCNTs. The black carbon was not monitored during the mist period, as the filter tape of the aethalometer was automatically advanced. In addition to the chemical composition, the structural properties of particles are helpful to anticipate the response of a specific particle size caused by CNTs. These properties can be ascertained based on a mass measurement of discretely singly-charged airborne particles using an aerosol particle mass analyzer (McMurry et al., 2002; Maynard et al., 2007).

TABLE 5
Time Course of Events for Metal Blending in Laboratory C

Time	Operation
12:00	Vacuum condition in metal blending equipment returned to atmospheric pressure for several hours after finishing metal blending process.
14:00	First opening of metal blending equipment. Furnace containing blended Al blocks and residual MWCNTs removed from metal blending equipment.
14:01	All contents of furnace poured onto flat tray on laboratory floor.
14:02	First closing of metal blending equipment.
14:07	Rotary-type oil vacuum pump started to simulate end step once again.
14:12	Mist filled room, presumably produced by rotary-type oil vacuum pump.
14:20	Inside of metal blending equipment became vacuum.
14:33	Pressure in metal blending equipment changed from vacuum to atmospheric pressure for several minutes.
14:34	Second opening of metal blending equipment.
14:36	Second closing of metal blending equipment.
14:45	Continuous monitoring of natural decay.
15:02	Termination of measurements.

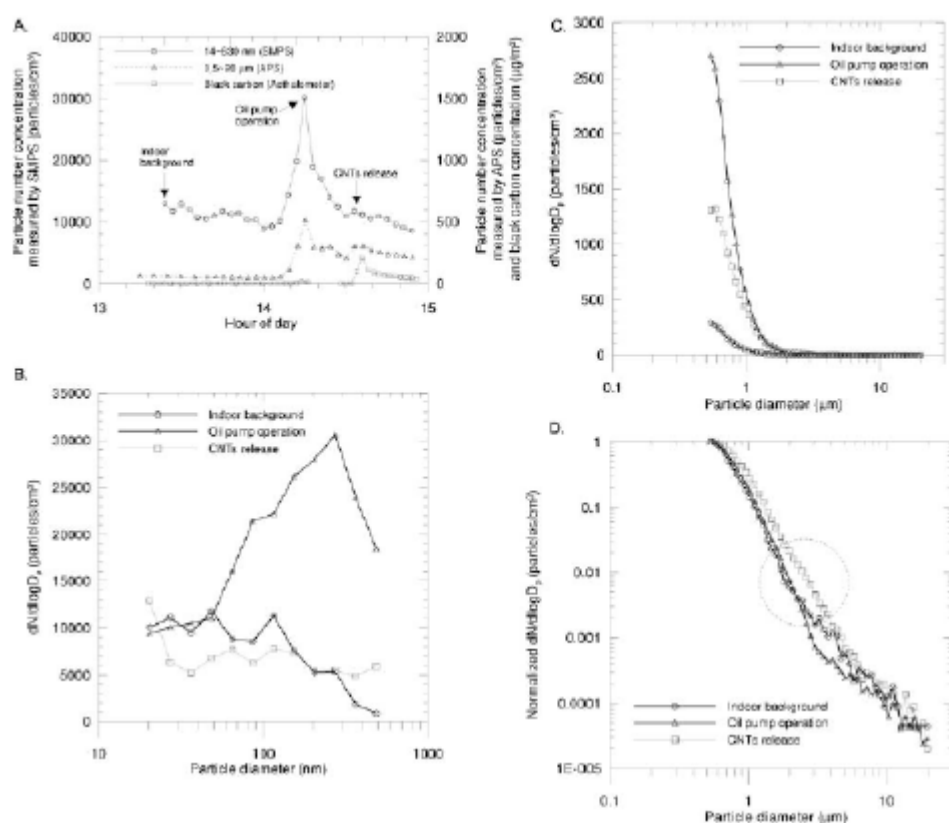


Figure 3. Number and size distributions of particles measured during blending operation in laboratory C. A. Size-specific particle numbers and black carbon concentrations measured using three real-time aerosol instruments; SMPS, APS and aethalometer; B. Particle size distributions measured using SMPS; C. Particle size distributions measured using APS; D. Normalized particle size distributions measured using APS. The dotted circle indicates the difference between the indoor background and those when opening the blending equipment.

Conclusions

The gravimetric concentrations of total dust before any control measures ranged from 0.21 to 0.43 mg/m^3 , then decreased to a non-detectable level after implementing the control measures. The number of MWCNTs in the samples obtained from the MWCNT blending laboratory ranged from 172.9-193.6 MWCNTs/cc before the control measures, and decreased to 0.018-0.05 MWCNTs/cc after the protective improvements. The real-time monitoring of aerosol particles provided a signature of the MWCNTs released from the blending equipment in laboratory C. In particular, the number size response of an aerodynamic particle sizer with a relatively high concentration in the range of 2 to 3 μm in aerodynamic diameter revealed the evidence of MWCNT exposure. The black carbon mass concentration also increased significantly during the MWCNT release process. Therefore, the present study suggests that the conventional industrial hygiene measures can significantly reduce exposure to airborne MWCNTs and other particulate materials in a nano research facility.

Reference

Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility, *Inhalation Toxicology*, 20: 741-749.

Test substances: MWCNTs at various manufacturing facilities

Methods

Sampling sites

The current study measured the nanoparticle concentrations inside various plants during July-September, 2008. The seven plants tested were mostly involved in MWCNT manufacturing and handling. The information related to each plant is shown in Table 1.

Personal and area sampling

The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8 μ m nominal pore-size, and 2 in. cowl, open-face) obtained from Pall Corp (P/N 64678, Michigan USA). The filter samples were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5-2.0 L/min. The sampling holders were also changed during the sampling period to avoid overload. The samplings were performed during the normal work period from 09:30 to 16:00. The typical duration of the air sampling lasted from 183 to 409 min. Some samples were also collected when placing the sampler 1-4 meters away from the manufacturing devices.

Real-time aerosol monitoring

An SMPS combining a differential mobility analyzer (Short type-DMA, 4220, HCT Co., Ltd, Korea; range 5–300 nm) and condensation particle counter (CPC, 4312, HCT Co., Ltd, 0–10⁸ particles/cm³ detection range) was used to monitor the particle size distribution with an electrical mobility diameter ranging from 14 to 685 nm. Meanwhile, a dust monitor (Model 1.109, Grimm) was used to observe the particle size distribution with a diameter ranging from 0.25 to 32 μ m. The total number concentration of particles larger than 3 nm was monitored by using an ultrafine particle counter (UCPC, 3025, TSI, 0-100,000 particles/cm³ detection range). In addition, a portable aethalometer (Model AE42-7-ER-MC, Magee Scientific) was utilized to measure the mass concentration of black carbon particles based on an optical absorption analysis. Here, the aethalometer sampled the total suspended particulate matter. The air in the laboratories was sampled at a flow rate of 0.3, 1.2, and 2 L/min for the SMPS, dust monitor, and aethalometer, respectively. The SMPS scanned the particle sizes at a time resolution of 3 min (120 s for up-scan and 30 s for retrace), while the average times for the dust monitor and aethalometer were 6 sec~5 min and 2 min, respectively.

MWCNT counting

The air samples were analyzed according to NIOSH analytical method 7402 (1994) and Han et al. (2008). The filter was coated with carbon and mounted onto carbon-coated copper grids (Veco, Eerbeek, Holland) using acetone vapor. Meanwhile, the CNTs were morphologically identified using a scanning transmission electron microscope (STEM, Hitachi 7100, Tokyo) and determined by comparing the elemental composition of the carbon nanotube using an energy dispersive X-ray analyzer (EDX, KEVEX 7000Q, Foster City, CA) (Han et al., 2008). All tubes and fiber structure aspect ratios greater than 3:1 were counted in more than 50 grid openings at random.

Results

CNT handling facilities

The CNT handling facilities were all involved in the manufacture or application of MWCNTs, where the application facilities were mainly research institutions using MWCNTs for research and development (Table 1).

Table 1. Information on workplaces.

Plant	Region (handling workers)	Manufactured materials	Process	Engineering controls	PPE use	Other process
A (Industry)	Asan (4)	Catalyst manufacturing, MWCNT manufacturing	Bag cyclone, CVD	Fume hood, enclosure local exhaust system	Half-mask, skin protective, glove	Preparation of CNT and catalyst
B (Industry)	Ulsan (3)	MWCNT, colloid manufacturing, lubricants	Electrolysis in water bath	Fume hood, enclosure local exhaust system	Working clothes	No
C (Industry)	Jeonju (5)	MWCNT manufacturing	CVD	Natural ventilation	No	Spray and filtration of CNT solution
D (Research Institute)	Jeonju (4)	MWCNT application	Ultrasonic dispersion, addition of H_2SO_4 in fume hood	Fume hood	Lab coat	No
E (Research Institute)	Daejeon (5)	MWCNT application	Spray of CNT solution, wafer heating	Fume hood, enclosed local exhaust system	Lab coat	CNT film on a filter
F (Lab)	Sewon (4)	MWCNT manufacturing	CVD	Fume hood	Lab coat	No
G (Lab)	Seoul (3)	MWCNT manufacturing, MWCNT application	CVD	Fume hood	Lab coat	No

Patterns of nanoparticle exposure

CVD manufacturing

Workplace C manufactured MWCNTs 5 times/day using the CVD method. The MWCNT manufacturing process is described in Figure 1. A time course of events is presented in Table 2. A filter sample obtained at area-6 (Figure 4 B) showed MWCNT tube structure (0.00312 tubes/cc) at TEM (Figure 2 A, Table 3) and small particles around the tube were mainly Fe by EDX analysis (Figure 2B). Filter samples collected by personal and area sampling ranged from 0.0311-0.2859 mg/m³ (Table 3). A sample collected on a TEM grid made of copper during 11:31~11:41 just after opening of CVD using an impactor showed no individual MWCNTs but clumped structures as shown in Figure 3 A. The source is thought to be related to release of metal catalysts such as iron and aluminum used in a CVD manufacturing as found from TEM-EDX analysis as shown in Figure 3 B and 3 C. Figure 4 A (a) is representative of the particle exposure in the MWCNT manufacturing workplaces, where most particles were emitted right after opening the CVD cover following MWCNT synthesis. The number concentration of particles larger than 0.25 μ m did not increase significantly right after opening of CVD because the sizes of released particles were less than 50 nm as shown in Figure 4 A (c). The increase in black carbon concentration was not much right after opening of CVD, implying that most of the released particles were not MWCNTs. From these results, the released particles when opening of CVD are considered to be mainly metal catalysts used for CNT manufacturing. Figure 4 B shows personal and area sampling locations plus SMPS and dust monitoring site.

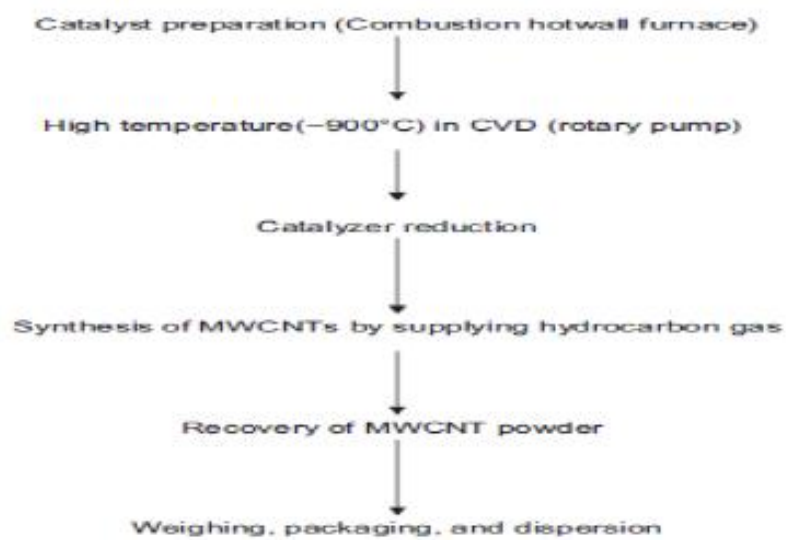


Figure 1. MWCNT manufacturing processes.

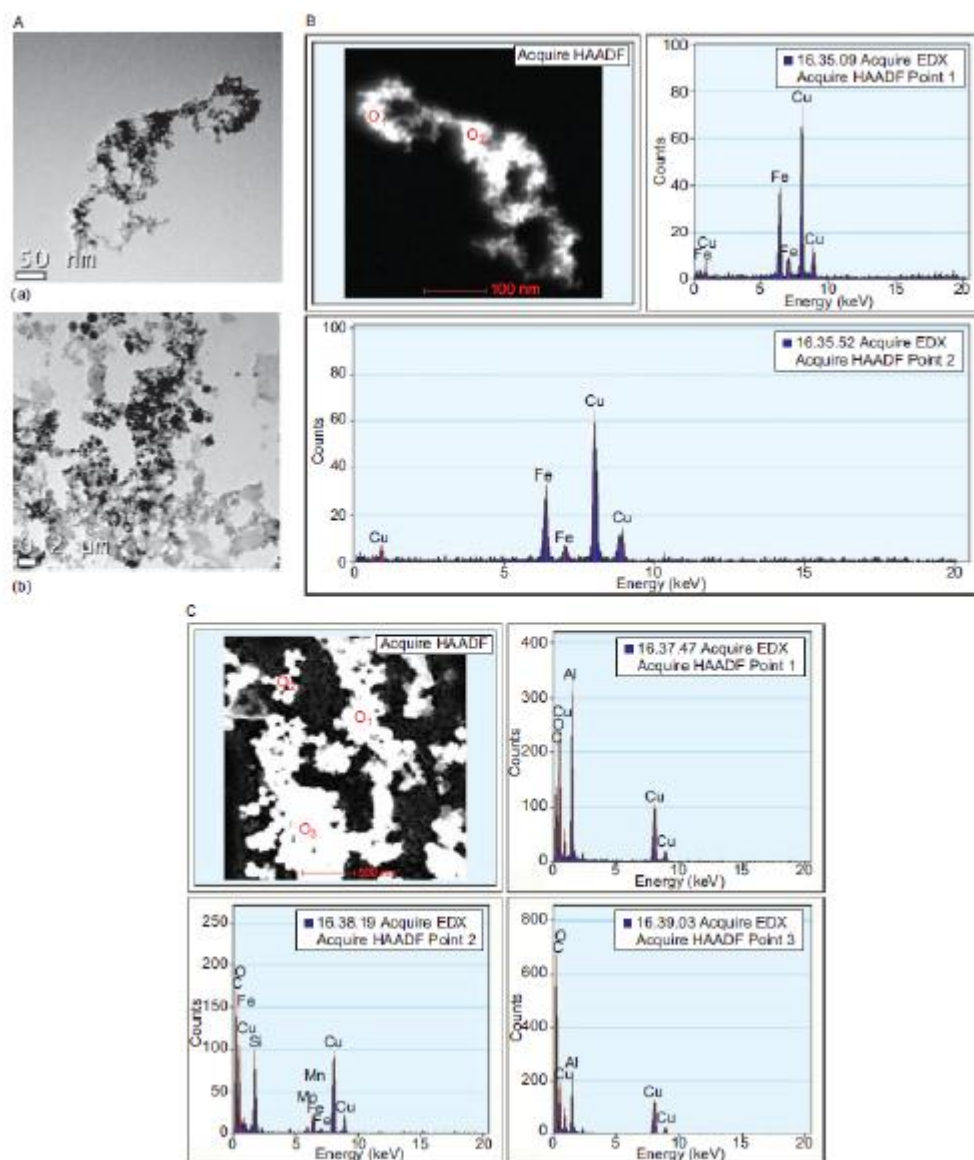


Figure 3. TEM micrograph of the grid sampled right after opening of CVD (11.31-11.41). (A) TEM image. (B) TEM-EDX analysis of (a). (C) TEM-EDX analysis of (b). (See colour version of this figure online at www.informahelthcare.com/ihc)

Table 2. Time course of events for MWCNT manufacturing in workplace C.

Time	Operation
10:58	Start of CNT solution spray
11:26	Opening of CVD and picking up the manufactured MWCNTs
11:29	Turning off CVD to clean
11:56	Weighing manufactured MWCNTs
11:57	Packing the manufactured MWCNTs into a zipper bag with breaking up some clumps
11:58	Vacuuming operation tables
12:02	Weighing catalysts CNT and placed in the CVD
12:15	Out of workplace for lunch
13:03	Start of work after lunch
13:04	Opening of CVD and picking up the manufactured MWCNTs, and cleaning of CVD
13:05	Operation of auto chiller
13:06	Closing of CVD and stopping of auto chiller
13:07	Translocation of the manufactured MWCNTs and vacuuming of tables
13:46	Packing the manufactured MWCNTs into a zipper bag with breaking up some clumps
13:49	Weighing catalysts CNT and placed in the CVD
14:36	Cooling of CVD and smoke can be seen due to cooling
14:37	Opening of CVD and picking up the manufactured MWCNTs,
14:38	Cleaning of CVD
14:41	Weighing catalysts CNT and placed in the CVD
14:41	Translocation of the manufactured MWCNTs
14:46	Weighing catalysts CNT and placed in the CVD
15:06	Packing the manufactured MWCNTs into a zipper bag with breaking up some clumps
15:18	Some operation using MWCNTs
15:54	Filtration of MWCNT solution into a flask

Table 3. Mass concentration of personal and area samples in workplace C.

	Filter weight (Before, mg)	Filter weight (After, mg)	Flow rate (L/min)	Sampling time (min)	Sampled volume (L)	Concentration (mg/m ³)	Number (tube/cc)
Personal 1	0.04243	0.04266	2.0028	358	717.0	0.2859	ND
Personal 2	0.04241	0.04261	2.0283	359	727.4	0.2406	N.D
Personal 3	0.03758	0.03763	2.0152	294	592.5	0.0422	ND
Area 5	0.03778	0.03785	1.9569	191	373.8	0.1204	ND
Area 6	0.03779	0.03786	1.9545	409	893.5	0.0560	0.00312
Area 7	0.03765	0.03770	1.9672	408	892.6	0.0311	ND
Area 8	0.03768	0.03774	1.9502	344	670.9	0.0522	ND

Note: ND, not detected.

CNT and catalyst preparation

Workplace A manufactured catalysts 1.2 t/year for CNT manufacture and also produced silica aerogel 0.5 t/year. At workplace A, catalysts were manufactured continually and CNT was manufactured once on the day of sampling. A time course of events is presented in Table 4. The workplace A was well equipped with engineering control such as fume hood to reduce exposure and bag cyclone and wet scrubber to protect environmental release. Workers were changed their clothing at the dress room and all process for CNT manufacturing were performed at well maintained environment. Filter samples collected by personal and area sampling ranged from 0.0177-0.1241 mg/m³ (Table 5). Figure 5 B shows personal and area sampling locations plus SMPS and dust

monitoring site. Figure 5 A shows the particle emission patterns during the CNT and catalyst preparation at workplace A. During preparation of MWCNTs into small bottles in a fume hood, the number concentration of particles larger than $0.25\ \mu\text{m}$ increased as average concentration $800\ \text{particles}/\text{cm}^3$ for the peak duration of 20 min. Vacuuming right after completion of the CNT preparation seems to be effective for reducing the particle number concentration measured by the dust monitor. This is thought to be one of the possible reasons why the increase in the black carbon concentration was not clearly observed during the CNT preparation.

Table 4. Time course of events in workplace A.

Time	Operation
10:30	Catalyst manufacturing start (Fe, Al, Mo mixed powder), initiation of personal and area sampling
13:04	Start of work after lunch
13:09	Start of changing catalyst container located in the right
13:25	Completion of changing catalyst container located in the right
13:36	Preparation of CNT into small bottles in a fume hood
13:49	Start of CVD
14:01	Vacuuming after completion of CNT distribution into small bottle in a fume hood
14:40	Vacuuming
14:50	Preparation of catalysts into small bottles with vacuum around container neck
15:01	Completion of preparing catalysts into small bottles. Vacuum is continually operating.
15:03	Turning off vacuum
15:44	Termination of catalyst manufacturing process. Scrubber is still operating.
15:55	Lowering temperature of CVD
16:00	Translocation of CNT manufacturing vessel. The door was not opened yet.
16:31	Termination of measurement

Table 5. Mass concentration of personal and area samples in workplace A.

	Filter weight (Before, mg)	Filter weight (After, mg)	Flow rate (L/min)	Sampling time (min)	Sampled volume (L)	Concentration (mg/m ³)	Number (tube/cc)
Personal 2	0.04230	0.04237	2.0270	236	470.6	0.0627	ND
Personal 3	0.04260	0.04268	2.0209	249	503.2	0.0795	ND
Personal 4	0.04288	0.04293	2.0327	235	470.6	0.0212	ND
Area 1	0.04283	0.04294	2.0293	278	564.1	0.1241	ND
Area 2	0.04270	0.04279	1.9918	274	545.8	0.0916	ND
Area 5	0.04234	0.04240	1.9719	290	571.9	0.0350	ND
Area 6	0.04265	0.04270	1.9982	282	563.5	0.0177	ND

Note: ND, not detected.

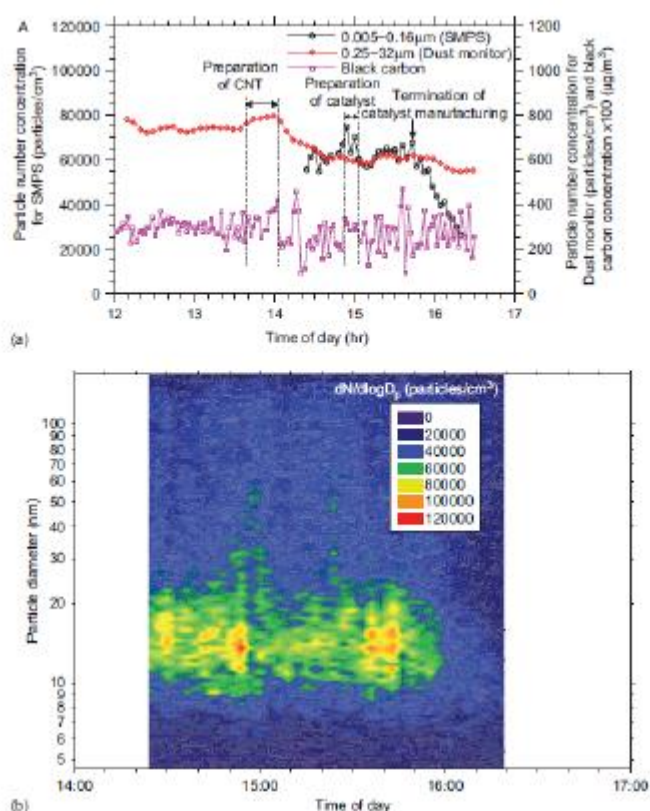
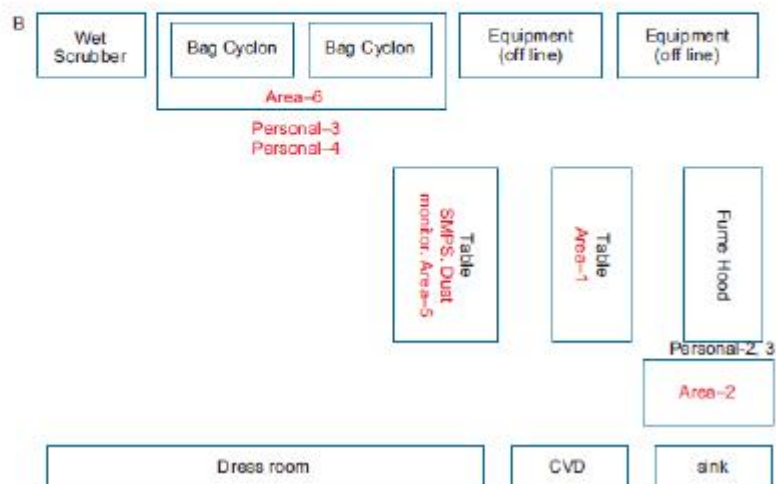


Figure 5. CNT and catalyst preparation (workplace A). (A) Real-time measurement: (a) SMPS, dust monitor and aethalometer; (b) change in particle size distributions measured by SMPS. (B) Process and sampling locations. (See colour version of this figure online at www.informahelthcare.com/ihx)

Figure 5. Continued.



During preparation of mixed metal catalysts into small bottles on the table where the real-time aerosol instruments were located, the number concentration of particles ranging from 5 nm to 160 nm in diameter that were measured by using the SMPS equipped with nano-DMA increased significantly as shown in Figure 5 A (a). The average number concentration of the particles with dominant sizes ranging from 10 nm to 20 nm was 75,000 particles/cm³ for the concentration peak that was caused by preparation of catalysts and lasted for approximate 10 min at workplace A (See Figure 5 A (b)).

Ultrasonic dispersion

Workplace D is a CNT application research facility. CNTs were weighed, sonicated and chemically treated with sulfuric acid in the fume hood at the day of measurement. The ultrasonic dispersion of CNT generated not only nanoparticles measured by the SMPS, but also fine particles detected by the dust monitor, as shown in Figure 6 A (a) because major sizes of the released particles were between 120 nm and 300 nm as shown in Figure 6 A (b). This large particle size released from the ultrasonic dispersion is different to that smaller than 100 nm for the released particles from the other processes measured in this study. Figure 6 B shows personal and area sampling locations plus SMPS and dust monitoring site. A time course of events is presented in Table 6. Filter samples collected by personal and area sampling ranged from 0.0078-0.1609 mg/m³ (Table 7).

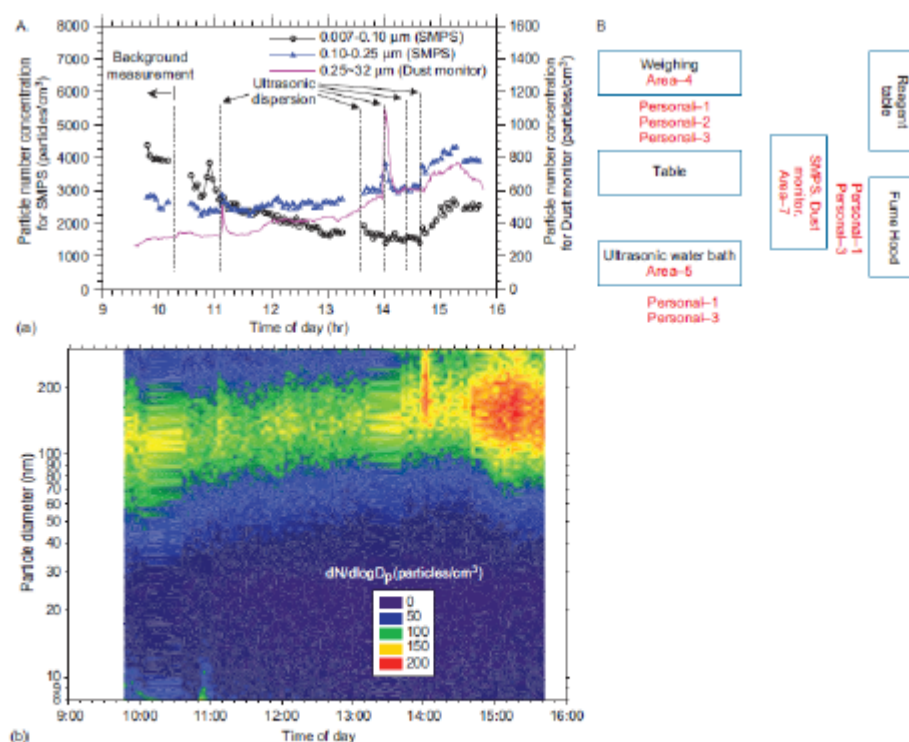


Figure 6. Ultrasonic dispersion (workplace D). (A) Real-time measurement: (a) SMPS and dust monitor; (b) change in particle size distributions measured by the SMPS. (B) Process and sampling locations. (See colour version of this figure online at www.informahelthcare.com/ihf)

Table 6. Time course of events in workplace D.

Time	Operation
09:47	Starting of background measurement
10:03	Weighing of CNT
11:06	Ultrasonic treatment
12:00	Lunch
13:36	Start of afternoon work, and ultrasonic treatment
14:00	Ultrasonic treatment
14:19	Termination of ultrasonic treatment
14:22	Turning on ultrasonic treatment
14:40	Addition of H_2SO_4 inside of fume hood, and turning on ultrasonic treatment
15:30	Termination of measurement

Table 7. Mass concentration of personal and area samples in workplace D.

	Filter weight (Before, mg)	Filter weight (After, mg)	Flow rate (L/min)	Sampling time (min)	Sampled volume (L)	Concentration (mg/m ³)	Number (tube/cc)
Personal 1	0.03768	0.03771	2.008	321	644.6	0.0078	ND
Personal 2	0.03774	0.03782	1.9612	302	592.3	0.0929	ND
Personal 3	0.03782	0.03793	1.9416	302	586.4	0.1450	ND
Area 4	0.04274	0.04288	1.9533	365	714.9	0.1609	ND
Area 5	0.04246	0.04255	1.9771	375	741.4	0.0877	ND

Note: ND, not detected.

Spraying CNTs onto the wafer and subsequent heating the wafer

Workplace E is a research facility to study applications of CNTs. CNTs were synthesized, separated and purified. CNTs were further dispersed with sonication, and the dispersed CNTs in solution were sprayed onto wafers to make CNT thin film in the enclosure that was equipped with ventilation fan at the date of measurement. Both of nanoparticles and fine particles that were generated while spraying were released when opening the CVD spray cover, as shown in Figure 7 A (a). The dominant particle sizes ranged from 50 nm to 110 nm as shown in Figure 7 A (b). The average number concentration of particles ranging from 0.014-0.25 μm and of particles ranging from 0.25-32 μm were 7606 particles/cm³ and 563 particles/cm³, respectively, during the concentration peak caused by CNT spraying. The peak of the black carbon concentration was too small and narrow to be considered as the response of CNTs as shown in Figure 7 A (a).

Heating of the wafer on which CNTs were coated by spraying was conducted for 10 minutes after opening the CNT spray cover when spraying of CNTs was completed at workplace E. During the heating process, the another peak with a small particle size smaller than 30 nm appeared in addition to the previous peak for opening of CNT spray cover as shown in Fig. 7 A (b). The average number concentrations of small particles ranging from 14 nm to 0.25 μm and of large particles ranging from 0.25 μm to 32 μm were 8966 particles/cm³ and 595 particles/cm³, respectively, for the concentration peak duration. Figure 7 B shows personal and area sampling locations plus SMPS and dust monitoring site. Filter samples collected by personal and area sampling ranged from 0.0804-0.1275 mg/m³ (Table 9). A time course of events is presented in Table 8. The TEM grid sampled for 10 minutes using an impactor before initiation of manufacturing process did not show CNTs but aggregates of metal components such as Fe and Mo as shown in Figure 8.

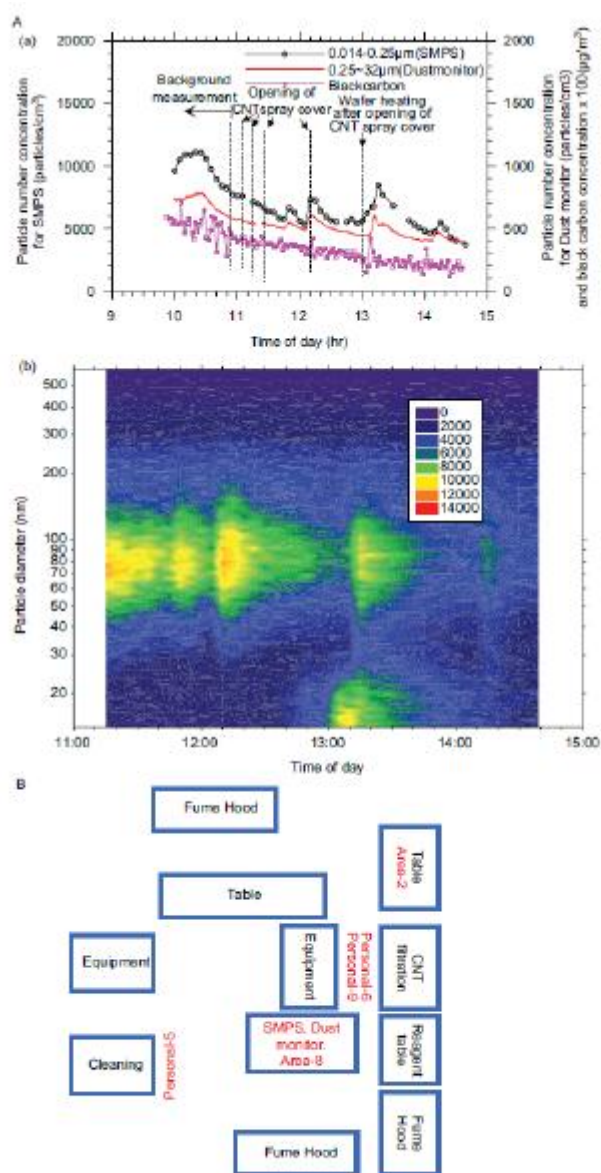


Figure 7. Spraying (workplace B). (A) Real-time measurement: (a) SMPS, dust monitor, and aethalometer; (b) change in particle size distributions measured by SMPS, inlet indicates $dN/d\log D_p$ (particles/cm³). (B) Process and sampling locations. (See colour version of this figure online at www.informabealthcare.com/ibt)

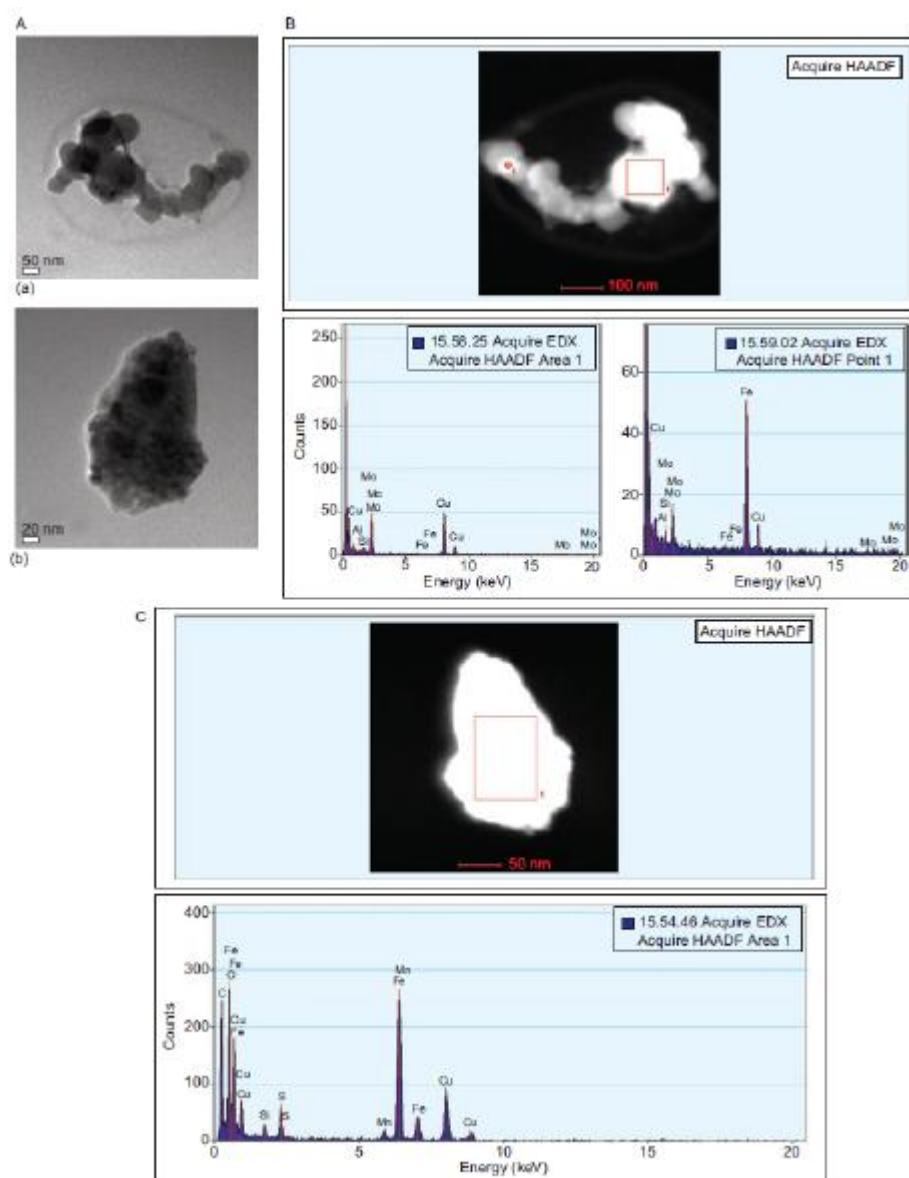


Figure 8. TEM micrograph of the grid sampled during background measurement (10:22-10:32). (A) TEM images. (B) TEM-EDX analysis of (a). (C) TEM-EDX analysis of (b). (See colour version of this figure online at www.informahelthcare.com/ht)

Table 8. Time course of events in workplace E.

Time	Operation
10:00	Starting of background measurement of laboratory located in the basement first floor
10:53	Starting of CNT spraying operation onto wafer after closing door of the enclosure equipped with a fan
11:06	CNT solution refill after opening of enclosure
11:12	CNT solution refill after opening of enclosure
11:20	CNT solution refill after opening of enclosure
12:10	CNT solution refill after opening of enclosure
12:15	Lunch
12:35	Spraying operation that was conducted in the enclosure was automatically terminated
12:50	Opening of the enclosure after lunch
13:00	Heating of the wafer from 80°C to 120°C for 10 minutes after opening of the enclosure
13:50	Starting of filtration to make CNT thin film
13:56	Drying of the CNT thin film in the oven after filtration
14:23	Opening of lab entrance

Table 9. Mass concentration of personal and area samples in workplace E.

	Filter weight (Before)	Filter weight (After)	Flow rate (L/min)	Sampling time (min)	Sampled volume (L)	Concentration (mg/m ³)	Number (tube/ml)
Personal 6	0.03714	0.03716	2.0189	183	369.459	0.1083	ND
Personal 5	0.03819	0.03821	1.9786	185	366.041	0.1083	ND
Personal 9	0.03734	0.03736	2.0643	219	452.082	0.0442	ND
Area 8	0.03706	0.03712	1.9876	314	627.246	0.1275	ND
Area 2	0.03789	0.03792	2.0006	311	622.187	0.0004	ND

Note: ND, not detected.

Exposure concentrations

The exposure concentrations of total suspended particulate matter ranged from 0.0078-0.3208 mg/m³ and 0.0126-0.1873 mg/m³ for the personal and area samplings, respectively (Table 10). As such, the concentrations were below the current ACGIH TLVs for carbon black (3.5 mg/m³) and PNOs (particles not otherwise specified, 3 mg/m³).

Table 10. Total suspended particle concentrations.

	Range (mg/m ³)	Mean (mg/m ³)	SD (mg/m ³)	GM (mg/m ³)	GSD
Personal sampling	0.0078-0.3208	0.1063	0.0877	0.0741	2.5966
Area sampling	0.0126-0.1873	0.0813	0.0459	0.0668	2.0256

Note: Mean, arithmetic mean; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

Release of nanoparticles during handling of MWCNTs

The release of nanoparticles during the handling of MWCNTs was also examined. As shown in Table 11, opening the CVD provided the highest frequency of nanoparticle release, followed by catalyst preparation. Other processes such as CNT preparation, opening the CNT spray cover, wafer heating, and opening the water bath also prompted nanoparticle release.

Table 11. Frequency of nanoparticle release during nanoparticle manufacturing.

Manufacturing	Process	Frequency of nanoparticle increase
CNT (N=7)	Opening of CVD	4
	Catalyst preparation	2
	CNT preparation	1
	Opening of CNT spray cover	1
	Wafer heating	1
	Opening of water bath	1
	Ultrasonic dispersion	1

Number and size of nanoparticles released during handling of CNTs

The number concentrations and size ranges of particles measured at seven workplaces were summarized in Table 12 by handling process types of MWCNTs. The catalyst preparation released nanoparticles with a mode diameter of 20-30 nm in the workplace environment so that their geometric mean concentration reached 37,350 particles/cm³ with a range of 18,600 – 75,000 particles/cm³. The CVD opening released also nanoparticles with a mode diameter of approximate 20 nm or 50 nm so that their geometric mean concentration increased to 11,039 particles/cm³ with a range of 6,974 - 16,857 particles/cm³. Although opening the CNT spray cover (7,606 particles/cm³), wafer heating (8,966 particles/cm³), and the ultrasonic operation (5,840 particles/cm³) released a similar number of nanoparticles ranging from 5,276-6,399 particles/cm³, the ultrasonic operation released larger particle sizes detectable with the SMPS and dust monitor when compared with the other operations.

Table 12. Number and size ranges of particles measured during CNT handling.

	Manufacturing Process	SMPS GM (range) (particles/cm ³)	Increasing particle size among SMPS data (nm)	Dust monitor GM (range) (particles/cm ³)
CNT (N=7)	Opening of CVD	11,039 (6,974–16,857)	50 and less, 20 and less	267 (152–432)
	Catalyst preparation	37,350 (18,600–75,000)	20–30	149
	CNT preparation	NM	NM	800
	Opening of CNT spray cover	7,606 (6,882–8,105)	50–110	563 (538–595)
	Wafer heating	8,966	30 and less	595
	Opening of Water bath	NM	NM	629 (581–670)
	Ultrasonic dispersion	5840 (5,276–6,399)	120–300	634 (511–1096)

Note. SMPS, particle number concentration in the SMPS size range (3–685 nm); Dust monitor, particle number concentration in the Dust Monitor size range (0.25–32 µm); GM, geometric mean; NM, not measured.

Conclusions

No workplace was found to exceed the current ACGIH TLVs (threshold limit values) and OELs (occupational exposure levels) set by the Korean Ministry of Labor for carbon black (3.5 mg/m³), PNOs (particles not otherwise specified, 3 mg/m³), and asbestos (0.1 fiber/cc). Nanoparticles and fine particles were most frequently released after opening the CVD (chemical vapor deposition) cover, followed by catalyst preparation. Other work processes that prompted nanoparticle release included spraying, CNT preparation, ultrasonic dispersion, wafer heating, and opening the water bath cover. All these operation processes could be effectively controlled with the implementation of exposure mitigation, such as engineering control, except at one workplace where only natural ventilation was used.

References

Exposure assessment of carbon nanotube manufacturing workplaces, *Inhalation Toxicology* 22(5):369-81