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**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**DOSSIER ON MULTIWALLED CARBON NANOTUBES (MWCNT)
- PART 2 -**

**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 2 -**



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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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; v) Mammalian Toxicology; and vi) Material Safety. 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. Kyunghee 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 and Industry Advisory Committee to OECD (BIAC).

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7. TOXICOLOGICAL INFORMATION

7.1 Toxicokinetics, metabolism and distribution

7.1.1 Basic toxicokinetics

Endpoint study record: Basic toxicokinetics.001 Grapjstrength C100 oral

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Result of EU Nanogenotox project

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Jacobsen et al.	2013	WP 7: Toxicokinetics and tissue distribution of MNs for specification of organs at risk for genotoxicity testing. Deliverable 7: Identification of target organs and biodistribution including ADME parameters.	EU 2nd Health Programme "Nanogenotox"					

Data access

data published

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
no guideline required		

Test materials

Test material identity

Identifier	Identity
	Arkema Graphistrength C100

Radiolabelling

yes

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: Rat Serum Albumin

Duration and frequency of treatment / exposure

single administration and repeated administration

Doses / concentrations

2.56 mg/ml

Dose amount : single administration; 10.9-11.3 mg/kg-bw for male and 10.9-11.3 mg/kg-bw for female

Repeated administration; 48-50 mg/kg-bw for male and 54.5-56.5 mg/kg-bw for female

No. of animals per sex per dose

18 male and 12 female

Control animals

yes, concurrent vehicle

Details on study design

In the frame of the Nanogenotox program, eighteen male (n=18) and 12 female (n=12) rats received a single (day 1) or repeated (on 5 consecutive days, day 1-5) oral administrations of ¹⁴C labelled Graphistrength C100 dispersed in 0.05 wt% Rat Serum Albumin (RSA) in ultra pure water. ¹⁴C-labelling of carbon nanotubes was carried out using a three steps chemical process [Georgin et al., 2009]. The single dose groups received a dose per animal between 9.6 – 10 mg/kg b.w. for male animals, and 10.9 – 11.3 mg/kg b.w. for female animals. After single treatment, radioactivity in tissues was evaluated in 3 male or female rats at days 2 and 90. Vehicle treated animals were investigated as follows; males n=2 at days 2, 6, and 90, and females n=1 at days 2, 6 and 90. Various organs and blood were collected including liver,

lung, spleen, kidney, heart and testes.

Results and discussions

Main ADME results

Type	
distribution	

Overall remarks, attachments

Remarks on results including tables and figures

After oral administration, ¹⁴C labelled MWCNTs didn't show translocation from the GI-tract into the systemic circulation or any of the organs investigated (including spleen, liver, and lung)

Endpoint study record: Basic toxicokinetics.002 Graphistrength C100 intravenous

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Result of EU Nanogenotox project

Data source

Data access

data published

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials

Test material identity

Identifier	Identity
	Arkem Graphistrength C100

Radiolabelling

yes

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Administration / exposure

Route of administration

intravenous

Vehicle

other: Rat Serum Albumin

Duration and frequency of treatment / exposure

single and repeated (5 consecutive days) administration

Doses / concentrations

2.56 mg/ml

Dose amount : single administration; 10.9-11.3 mg/kg-bw for male and 10.9-11.3 mg/kg-bw for female

Repeated administration; 48-50 mg/kg-bw for male and 54.5-56.5 mg/kg-bw for female

No. of animals per sex per dose

18 male and 12 female

Control animals

yes, concurrent vehicle

Details on study design

In the frame of the Nanogenotox program, eighteen male (n=18) and 12 female (n=12) rats received a single (day 1) or repeated (on 5 consecutive days, day 1-5) intravenous administrations of ¹⁴C labelled Graphistrength C100 dispersed in 0.05 wt% Rat Serum Albumin (RSA) in ultra pure water. ¹⁴C-labelling of carbon nanotubes was carried out using a three steps chemical process [Georgin et al., 2009]. The repeated dose groups received a total cumulative dose per animal between 48 – 50 mg/kg b.w after 5 days treatment, for male animals, and 54.5 – 56.5 mg/kg b.w. for female animals. Tissue samples were obtained at various time points after the administration. Repeated treatments tissue levels were evaluated for 3 males at days 6, 14, 30 and 90, and for females at days 6 and 90. Vehicle treated animals were investigated as follows; males n=2 at days 2, 6, and 90, and females n=1 at days 2, 6 and 90. Various organs and blood

were collected including liver, lung, spleen, kidney, heart and testes.

Results and discussions

Main ADME results

Type
distribution

Bioaccessibility

Any other information on results incl. tables

In blood obtained at 24 hours after the intravenous administration of the ¹⁴C-**Graphistrength C100** very low to almost no radioactivity was detected whatever the protocol of administration in either male or female rats.

After a single intravenous dose of ¹⁴C-**Graphistrength C100** to male rats only 8% of the injected dose was observed in organs at day 1, with the liver being the main target organ (7%). In females the recovery was 24% after a single dose. At day 90 a 50% reduction of the level of radioactivity was observed when compared to day 1 in most organs, while for the lung a similar % of injected dose was observed both on day 1 after a single administration and at day 90 after repeated administration. After the repeated intravenous dosing for male rats compared to the data of day 6, almost no elimination of ¹⁴C-**Graphistrength** was observed at day 90 after administration. However, when comparing the elimination after repeated exposures and single exposure, ¹⁴C-**Graphistrength C100** elimination appeared much more reduced. After a single exposure a 50% elimination was observed while almost no elimination was observed after repeated intra doses. A particular feature with this ¹⁴C-**Graphistrength C100** was the observation that spleen was more loaded than lung at any time point.

The MWCNTs were found to display particular bioaccumulation and biopersistence properties in the various organs evaluated.

Table 6-2A, % of injected dose in organs in rat males (single dose)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
IV NM 400	1,92 ± 0,33	23,79 ± 3,89	24,82 ± 1,76	1,89 ± 0,96	0,00	0,24 ± 0,04	0,00	0,22 ± 0,05		12,5 ± 2,5	65% ± 9%
IV NM 401	0,52 ± 0,42	15,11 ± 6	1,78 ± 1,5	0,37 ± 0,3	0,00	0,00	0,00	0,00	0,04 ± 0,01	0,00	17,8% ± 8%
Graphistrength C100	0,55 ± 0,14	6,98 ± 0,64	0,27 ± 0,13	0,07 ± 0,04	0,00	0,00	0,00	0,00		0,3 ± 0,3	8,2% ± 1,2%
IV NM NRCWE-006	1 ± 0,54	28,21 ± 8,37	1,2 ± 0,65	0,25 ± 0,04	0,00	0,00	0,00	0,16 ± 0,74	0,32 ± 0,19	0,00	31,1% ± 10,5%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
IV NM 400	0,39 ± 0,25	8 ± 1,83	11,4 ± 2,8	0,00	0,00	0,00	0,00	0,00	0,24 ± 0,15	0,00	20% ± 5%
IV NM 401	0,38 ± 0	3,46 ± 0,42	0,59 ± 0,17	0,13 ± 0,1	0,00	0,00	0,00	0,05 ± 0,04	0,09 ± 0,004	0,00	4,7% ± 0,7%
Graphistrength C100	0,23 ± 0,13	3,15 ± 2,2	0,3 ± 0,11	0,00	0,00	0,00	0,00	0,00		0,00	3,7% ± 2,4%
IV NM NRCWE-006	1 ± 0,83	16,40 ± 4,6	1 ± 0,73	0,22 ± 0,06	0,00	0,00	0,00	0,33 ± 0,21	2,12 ± 2	0,00	21% ± 8,4%

Table 6-2B, % of injected dose in organs in rat females (single dose)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	0,69 ± 0,09	24,93 ± 14,6	1,15 ± 0,52	0,59 ± 0,35	0,00	0,00	0,00	0,00		10,8 ± 0,94	38,2% ± 16,6%
IV NM 401	0,22 ± 0,14	6,37 ± 3,87	1,03 ± 0,75	0,28 ± 0,02	0,00	0,00	0,00	0,02 ± 0,02	0,01 ± 0,6	0,00	7,9% ± 5,4%
Graphistrengt C100	0,62 ± 0,33	23 ± 5	0,54 ± 0,19	0,08 ± 0,04	0,00	0,00	0,00	0,00		0,44 ± 0,03	24,6% ± 5,6%
IV NM NRCWE-006	0,85 ± 0,56	35,6 ± 18,2	2,17 ± 1,27	0,26 ± 0,21	0,00	0,00	0,05 ± 0,05	0,00	0,026 ± 0,02	2,73 ± 0,96	42% ± 21%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Ovaries	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	0,36 ± 0,24	3,4 ± 0,61	8,2 ± 0,56	0,00	0,00	0,00	0,00	0,00	0,09 ± 0,04	0,00	12% ± 1,5%
IV NM 401	0,27 ± 0,11	2,5 ± 0,93	0,7 ± 0,28	0,18 ± 0,13	0,00	0,00	0,00	0,00	0,04 ± 0,15	0,00	3,7% ± 1,6%
Graphistrengt C100	0,57 ± 0,14	5,5 ± 1,1	0,54 ± 0,25	0,00	0,00	0,00	0,00	0,00		0,00	6,6% ± 1,5%
IV NM NRCWE-006	1,32 ± 0,63	17,30 ± 7,08	1,83 ± 0,56	0,28 ± 0,17	0,00	0,00	0,00	0,00	0,11 ± 0,23	0,00	20,8% ± 8,7

Table 6-3A: % of injected dose in organs in rat males (repeated doses)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	2,35 ± 0,39	25,42 ± 3,80	4,40 ± 4	1,30 ± 0,63	0,00	0,17 ± 0,10	0,07 ± 0,01	0,27 ± 0,09		5,17 ± 2,1	39% ± 10 %
IV NM 401	0,3 ± 0,04	3,96 ± 1,2	1,05 ± 1	0,10 ± 0,07	0,00	0,00	0,00	0,06 ± 0,03	0,10 ± 0,60	0,00	5,6% ± 3 %
Graphistrengt C100	0,25 ± 0,06	4,78 ± 1,8	0,11 ± 0,03	0,02 ± 0,005	0,00	0,00	0,00	0,00		0,14 ± 0,03	5,3% ± 1,9%
IV NM NRCWE-006	0,71 ± 0,5	22,96 ± 18,8	1,56 ± 1,2	0,51 ± 0,21	0,00	0,07 ± 0,06	0,00	0,00	0,04 ± 0,06	0,25 ± 0,04	26,1% ± 20,5%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Ovaries	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	2,20 ± 1,22	35,21 ± 15,6	4,42 ± 3,26	0,43 ± 0,26	0,00	0,00	0,1 ± 0,04	0,09 ± 0,05		2,12 ± 0,95	44,6% ± 21%
IV NM 401	0,58 ± 0,22	5,82 ± 0,97	0,41 ± 0,14	0,07 ± 0,04	0,00	0,00	0,00	0,00	0,01 ± 0,007	0,00	6,9 % ± 1,5%
Graphistrengt C100	0,30 ± 0,03	4,29 ± 0,4	0,1 ± 0,04	0,01 ± 0,006	0,00	0,00	0,00	0,02 ± 0,01		0,04 ± 0,02	4,7% ± 0,5%
IV NM NRCWE-	1,57 ± 0,26	34,5 ± 5	1,9 ± 0,48	0,19 ± 0,03	0,00	0,00	0,00	0,00	0,22 ± 0,01	0,00	38,4% ± 5,8%

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Table 6-3B,% of injected dose in organs in rat females (repeated doses)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	2,26 ± 0,6	34,94 ± 16,02	32,78 ± 11,21	1,30 ± 0,46	0,00	0,28 ± 0,09	0,07 ± 0,02	0,02 ± 0,004	5,87 ± 0,40		77,3% ± 28,8%
IV NM 401	0,13 ± 0,1	4,1 ± 0,77	0,55 ± 0,026	0,09 ± 0,07	0,00	0,03 ± 0,02	0,00	0,006 ± 0,003	0,00	0,005 ± 0,002	5% ± 0,9%
Graphistrengt C100	0,42 ± 0,19	10,59 ± 5	0,40 ± 0,4	0,04 ± 0,02	0,00	0,00	0,00	0,00	0,16 ± 0,30		11,6% ± 5,9%
IV NM NRCWE-006	0,76 ± 0,4	34,76 ± 20,1	2,42 ± 2	0,19 ± 0,15	0,00	0,06 ± 0,03	0,00	0,01 ± 0,004	0,30 ± 0,09	0,08 ± 0,03	38,6% ± 22,7%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Ovaries	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	0,21 ± 0,13	7,92 ± 2,46	4,68 ± 0,97	0,04 ± 0,01	0,00	0,00	0,00	0,00	0,00	0,12 ± 0,06	13% ± 3,6%
IV NM 401	0,33 ± 0,05	2,7 ± 0,39	0,39 ± 0,06	0,05 ± 0,01	0,00	0,00	0,00	0,00	0,00	0,1 ± 0,1	3,6% ± 0,6%
Graphistrengt C100	0,41 ± 0,02	3,21 ± 2,79	0,18 ± 0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00	3,8% ± 2,9%
IV NM NRCWE-006	1,82 ± 0,55	22,02 ± 6,88	2,28 ± 0,95	0,31 ± 0,11	0,00	0,00	0,00	0,00	0,00	0,58 ± 0,34	27% ± 8,8%

Endpoint study record: Mitsui MWNT-7: Basic toxicokinetics: 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	Aiso, S. et al.	2011	Translocation of Intratracheally Instilled Multiwall Carbon Nanotubes to Lung-Associated Lymph Nodes in Rats	Industrial Health, 49, 215-220 (2011)					

Data access

data published

Materials and methods**Type of method**

in vivo

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 MWNT-7

Radiolabelling

no

Test animals**Species**

rat

Strain

Fischer 344

Sex

male

Administration / exposure**Route of administration**

intratracheal

Vehicle

other: Phosphate-buffered saline (PBS) containing 0.1% Tween 80

Duration and frequency of treatment / exposure

single intratracheal instillation

Doses / concentrations

0 (vehicle), 40 and 160 µg/rat

No. of animals per sex per dose

8 rats per dose per each time point were sacrificed. Three doses and 4 time points were used.

Control animals

yes

Any other information on materials and methods incl. tables

The test substance was suspended in phosphate-buffered saline (PBS) containing 0.1% Tween 80 as a colloidal dispersant and subjected to ultrasonication for 20 min with an ultrasonic homogenizer.

At the age of 13 wk, male F344 rats were intratracheally instilled with MWCNT at doses of 0 (vehicle), 40 or 160 µg/rat.

The rats were sacrificed on days 1, 7, 28 or 91 after instillation and light microscopic examinations were performed on lung-associated lymph nodes (LALN) tissues.

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

MWCNT was translocated to right and left posterior mediastinal lymph nodes and parathymic lymph nodes. Deposition of MWCNT was greater in the posterior mediastinal lymph node than in the parathymic lymph node, and the amount of MWCNT deposited in these two lymph nodes increased gradually and dose-dependently with time during the 91-d post exposure period. MWCNT was phagocytosed by nodal macrophages, and some of the MWCNT-laden macrophages were aggregated.

TEM observation confirmed that in the cytoplasm of MWCNT-laden, nodal macrophages there were fibers having multi-layered cylindrical structures of 35.5 or 53 nm in width, which were characteristic of MWCNT morphology.

Overall remarks, attachments**Attached full study report**

Attached full study report
Mitsui MWNT-7 ADME Industrial Health 2011, 49, 215-220.pdf / 1.78 MB (application/pdf)

Translocation of Intratracheally Instilled Multiwall Carbon Nanotubes to Lung-Associated Lymph Nodes in Rats

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Abstract: In order to assess the extrapulmonary effects of multiwall carbon nanotubes (MWCNT), deposition of MWCNT and histopathologic changes in lung-associated lymph nodes (LALN) were examined in MWCNT-administered rats. At the age of 13 wk, male F344 rats were intratracheally instilled with MWCNT at a dose of 0 (vehicle), 40 or 160 $\mu\text{g}/\text{rat}$. The rats were sacrificed on Day 1, 7, 28 or 91 after instillation and light microscopic examinations were performed on LALN tissues. MWCNT was translocated to right and left posterior mediastinal lymph nodes and parathymic lymph nodes. Deposition of MWCNT was greater in the posterior mediastinal lymph node than in the parathymic lymph node, and the amount of MWCNT deposited in these two lymph nodes increased gradually and dose-dependently with time. MWCNT was phagocytosed by nodal macrophages, and some of the MWCNT-laden macrophages were aggregated. Transmission electron microscopic (TEM) observation confirmed the presence of MWCNT fibers with a characteristic multi-walled cylindrical structure.

Key words: Multiwall carbon nanotube, Lung-associated lymph node, Mediastinal lymph node, Macrophages, Granuloma

Rapid development of the carbon nanotube (CNT) industry and extensive industrial applications of CNTs in various sectors of industry have raised serious concerns over health risks of workers exposed to CNTs. Neither epidemiological nor medical case studies have been reported on health outcomes of CNT-exposed workers. Recent *in vivo* toxicity studies have shown induction of mesotheliomas after intraperitoneal injection of multi-wall carbon nanotube (MWCNT) in *p53* gene-deficient mice¹⁾ and asbestos-like pathogenicity after intraperitoneal injection of MWCNT in female mice²⁾. Asbestos fibers are known to cause mesotheliomas in exposed

workers³⁾ and animals⁴⁾, and have been reported to migrate to the serosal tissues in insulation workers with long-term asbestos exposures⁵⁾, and to translocate to the pleural cavity in rats administered chrysotile fibers by intratracheal instillation⁶⁾. We found in a previous study⁷⁾ that intratracheal instillation of MWCNT in male rats increased the deposition of MWCNT dose- and time-dependently in the bronchus-associated lymphoid tissue (BALT) which is anatomically enclosed within the lung. However, whether or not BALT is involved in the pulmonary clearance of particles is still unclear⁸⁾. On the other hand, lung-associated lymph nodes (LALN) and their afferent and efferent lymphatic pathways have been described morphologically⁹⁻¹⁰⁾, and are recognized as a structure important for lung defense and the sys-

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temic immune system⁸⁾. The right and left posterior mediastinal lymph nodes and the parathymic lymph node have different lymphatic drainage pathways to clear particles from the lung^{9, 10)}. Recent rodent studies have addressed extrapulmonary effects of MWCNT on the systemic immune function^{11, 12)}. These extrapulmonary effects might be mediated in part through the translocation of MWCNT to the LALN¹³⁾, and finally to the general circulation, since pulmonary lymphatic ducts from the posterior mediastinal and parathymic lymph nodes drain particles into the blood stream at the subclavian vein^{9, 10)}.

This short communication reports the time-course changes in the deposition of MWCNT and MWCNT-induced histopathologic changes in the right and left posterior mediastinal lymph nodes and parathymic lymph node of male rats that received intratracheal instillation of MWCNT.

Male F344/DuCr1Cr1j rats were purchased from Charles River Japan, Inc. (Kanagawa, Japan) at the age of 11 wk. The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals¹⁴⁾, and the present study was approved by the ethics committee of the Japan Bioassay Research Center.

MWCNT was kindly supplied by MITSUI & Co. Ltd (MWNT-7, Lot No. 061220, Tokyo, Japan). The test substance was suspended in phosphate-buffered saline (PBS) containing 0.1% Tween 80 as a colloidal dispersant and subjected to ultrasonication for 20 min with an ultrasonic homogenizer (VP-30S, 20 kHz, 300 W, TAITEC Co., Ltd, Tokyo, Japan). Immediately before intratracheal instillation, the ultrasonicated suspension of MWCNT or the vehicle solution was subjected to additional ultrasonication for 30 s with a sonicator (US-2, As One Co., Ltd., Tokyo, Japan).

The suspension of MWCNT in the PBS-Tween 80 or the vehicle solution was intratracheally instilled in rats after inhalational anesthetization with isoflurane gas (Forane, Abbott Japan Co., Ltd., Tokyo, Japan). Experimental groups received MWCNT at a dose of 0 (vehicle), 40 or 160 $\mu\text{g}/\text{rat}$, as described in our previous study⁷⁾. MWCNT- and vehicle-dosed rats were sacrificed on Day 1, 7, 28 or 91 following instillation.

Eight rats/group were sacrificed at each time point by exsanguination from the jugular vein under pentobarbital anesthesia, and the trachea was ligated. In preparation for light microscopic examination, organs and tissues were fixed by perfusion with physiological saline and subsequently 10% neutral buffered formalin. All posterior mediastinal and parathymic lymph nodes were removed together with the trachea and thymus, and

embedded in paraffin. About 5 μm -thick slices of right and left posterior mediastinal and parathymic lymph nodes were sectioned, and stained with hematoxylin and eosin (H & E).

Deposition of MWCNT was semi-quantitatively evaluated for the extent of instilled MWCNT deposition in the LALN tissues. The extent of MWCNT deposition or the severity of histopathologic change was scored by microscopic observation of H & E-stained tissues, according to the following criteria. Score 1, termed "slight", indicates that slight MWCNT deposition or histopathologic change was observed in a limited part of the area. Score 2, termed "moderate", indicates that slight MWCNT deposition or histopathologic change was observed in a large part of the area or that moderate MWCNT deposition or histopathologic change was observed in a limited part of the area. Score 3, termed "marked", indicates that moderate MWCNT deposition or histopathologic change was observed in a large part of the area or that marked MWCNT deposition or histopathologic change was observed in a limited part of the area.

The right posterior mediastinal lymph node of a rat receiving MWCNT at a dose of 160 μg and sacrificed on Day 91 following instillation was examined by transmission electron microscopic (TEM) observation for MWCNT fibers (JEM-1400, JEOL, Tokyo, Japan). The lymph node was fixed with 0.074 M phosphate-buffer solution containing 2% paraformaldehyde and 1.0% glutaraldehyde and post-fixed with 1% osmium tetroxide. Thereafter, the lymph node was dehydrated in graded ethanol, and embedded in epoxy resin. The ultrathin sections were stained with uranyl acetate and lead citrate and used for the TEM observation.

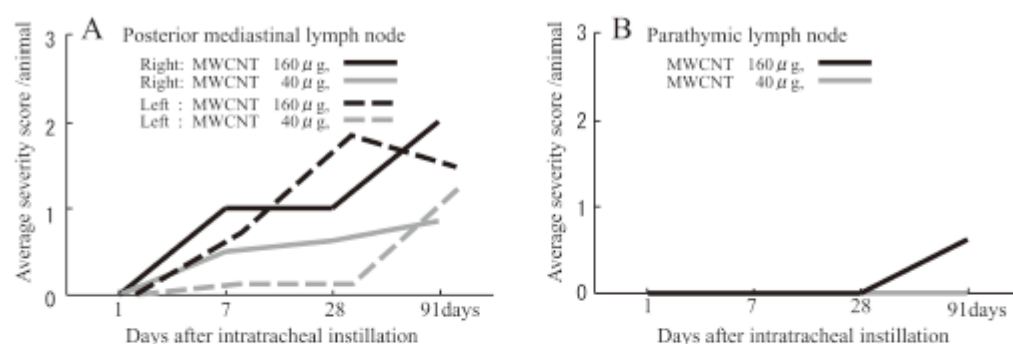
Table 1 shows the deposition of MWCNT and the extent of MWCNT deposition in the right and left posterior mediastinal lymph nodes and in the parathymic lymph node as well as their MWCNT-induced histopathologic changes, i.e., aggregates of MWCNT-laden macrophages, on different days after instillation. Figure 1 also shows the time-course changes in the extent of MWCNT deposition in these lymph nodes expressed as the severity score averaged over the number of animals examined. No MWCNT fibers were found in the lymph nodes of 40 or 160 μg -dosed rats on Day 1. In the right and left posterior mediastinal lymph nodes, slight deposition of MWCNT fibers occurred on Day 7, and the incidence and severity of the MWCNT deposition increased dose- and time-dependently during the post-administration period after Day 7. In the parathymic lymph node, however, deposition of MWCNT fibers occurred only in three out of eight 160 μg -dosed rats on Day 91. Light-microscopic examination revealed

Table 1. Deposition of intratracheally instilled MWCNT and histopathologic changes in LALN at a dose of 0 (vehicle), 40 or 160 μg in the rats sacrificed on different days after instillation

Dose of MWCNT (/rat) Days after instillation	0 μg				40 μg				160 μg			
	1	7	28	91	1	7	28	91	1	7	28	91
Posterior mediastinal lymph node, right												
<No. of rats examined>	<6>	<2>	<8>	<8>	<3>	<4>	<8>	<7>	<5>	<4>	<7>	<8>
MWCNT deposition ¹	0	0	0	0	0	2	5	4	0	4	5	8
(Slight)						(2)	(5)	(2)		(4)	(3)	(0)
(Moderate)								(2)			(2)	(8)
Aggregate of MWCNT-laden macrophages	0	0	0	0	0	0	0	2	0	0	0	6
Posterior mediastinal lymph node, left												
<No. of rats examined>	<7>	<8>	<8>	<8>	<7>	<8>	<8>	<8>	<8>	<8>	<7>	<8>
MWCNT deposition	0	0	0	0	0	1	1	7	0	6	7	7
(Slight)						(1)	(1)	(4)		(6)	(1)	(2)
(Moderate)								(3)			(6)	(5)
Aggregate of MWCNT-laden macrophages	0	0	0	0	0	0	0	2	0	0	0	5
Parathymic lymph node												
<No. of rats examined>	<8>	<7>	<8>	<8>	<8>	<7>	<8>	<8>	<8>	<7>	<8>	<8>
MWCNT deposition	0	0	0	0	0	0	0	0	0	0	0	3
Slight												(1)
Moderate												(2)
Aggregate of MWCNT-laden macrophages	0	0	0	0	0	0	0	0	0	0	0	2

¹MWCNT fibers were engulfed by macrophages.

Because of failure in sampling the right posterior mediastinal lymph node, the right side of several rats was not available for histopathological analysis.

**Fig. 1.** Time-course changes in the extent of which MWCNT deposition in the right and left posterior mediastinal (A) and parathymic lymph (B) nodes of rats which received intratracheal instillation of MWCNT.

that macrophages were present in both the mediastinal and parathymic lymph nodes. Nodal macrophages were less abundant than alveolar macrophages in the alveolar space and wall, which we observed in the previous study⁷⁾. As indicated by arrows in Fig. 2, dispersed MWCNT fibers engulfed by macrophages were observed in the lymph nodes, and some of the macrophages were filled with MWCNT fibers, the extent of which was scored as moderate. Small aggregates of several MWCNT-laden macrophages were focally

formed on Day 91 in both the posterior mediastinal lymph nodes of 40 and 160 μg -dosed rats and in the parathymic lymph node of 160 μg -dosed rats, as indicated by the asterisks in Fig. 2. However, development of aggregated MWCNT-laden macrophages to granulomas and associated inflammation was not evident in any of the mediastinal or parathymic lymph nodes of the 40 or 160 μg -dosed rats throughout the 91-d postexposure period.

As shown in Fig. 3, TEM observation of the right

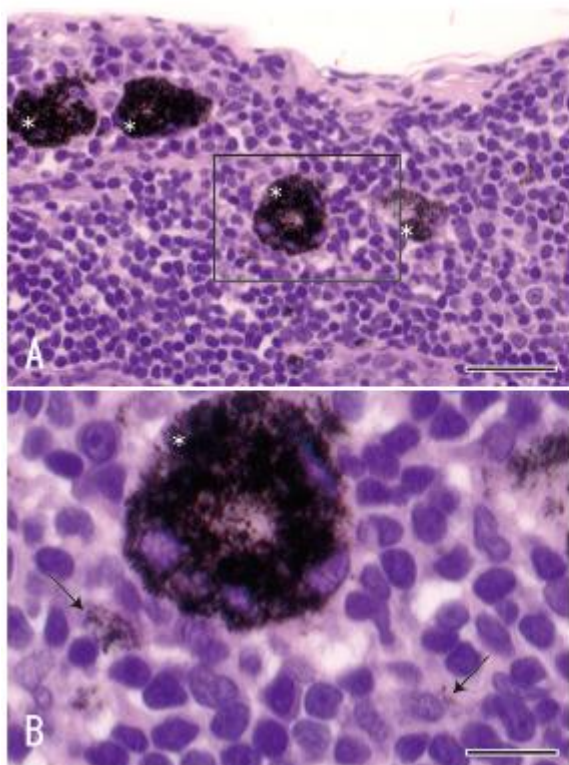


Fig. 2. Deposition of MWCNT in the right posterior mediastinal lymph node of a rat which received intratracheal instillation of MWCNT at a dose of 160 μg and was sacrificed on Day 91 after instillation.

In A, small aggregates of several MWCNT-laden macrophages are indicated by asterisks. H & E stain. Bar indicates 50 μm . In B, a magnified image of the enclosed area shown in A. Arrows indicate MWCNT fibers engulfed by macrophages. Bar indicates 17 μm .

posterior mediastinal lymph node revealed that fibers having multi-layered cylindrical structures of 35.5 or 53 nm in width, which were characteristic of MWCNT morphology, were present in the cytoplasm of nodal macrophages.

In the present study, deposition of intratracheally instilled MWCNT in both the right and left posterior mediastinal lymph nodes of rats was found to increase gradually and dose-dependently during the 91-d post-exposure period, and MWCNT fibers focally formed small aggregates of several MWCNT-laden macrophages only on Day 91 after instillation. Ma-Hock *et al.*¹³⁾ reported deposition of MWCNT in the mediastinal lymph node of rats exposed by inhalation to MWCNT aerosol. The translocation of MWCNT to the mediastinal lymph node found in the present study is comparable with the findings of Ma-Hock *et al.*¹³⁾ that 3-month inhalation

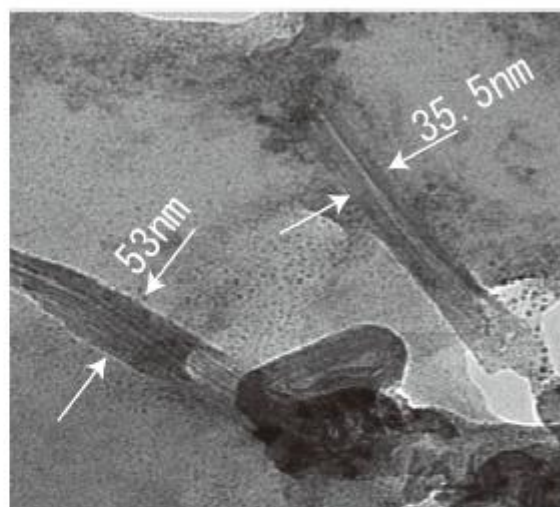


Fig. 3. A TEM image of MWCNT in the right posterior mediastinal lymph node of a rat which received intratracheal instillation of MWCNT at a dose of 160 μg and was sacrificed on Day 91 after instillation.

exposure of rats to MWCNT aerosol at 0.1–2.5 mg/m^3 induced granulomatous inflammation and lymphoreticulo-cellular hyperplasia in the mediastinal lymph node as well as particle-laden macrophages. The principal difference in the character of the MWCNT-induced lesions in the mediastinal lymph node between the two studies is the slight formation of aggregated, MWCNT-laden macrophages in the present study, as compared with induction of granulomas and associated inflammation reported by Ma-Hock *et al.*¹³⁾. This difference might be due to the difference in amount of pulmonary deposition of MWCNT. Ma-Hock *et al.*¹³⁾ estimated that 46.8, 243 and 1,170 μg of MWCNT per lung were deposited in the lung after 90-d inhalation exposure to 0.1, 0.5 and 2.5 mg/m^3 , respectively, while 40 and 160 μg per lung were intratracheally instilled in the present study. These values indicate that amount of MWCNT depositing in the pulmonary region was much greater in the study of Ma-Hock *et al.*¹³⁾ than in the present study. The small aggregates of several MWCNT-laden macrophages in the LALN were much smaller than the microgranulomas found in a previous study, which had diameters up to 150 μm in the alveolar wall and were composed of the MWCNT-laden alveolar macrophages⁷⁾. It can be inferred, therefore, that small aggregations of MWCNT-laden macrophages progress to from microgranulomas and associated inflammation in the LALN when the dose level of MWCNT is increased.

TEM observation confirmed that in the cytoplasm of MWCNT-laden, nodal macrophages there were fibers

having multi-layered cylindrical structures of 35.5 or 53 nm in width, which were characteristic of MWCNT morphology^{12, 15).}

Increased deposition of MWCNT in the parathymic lymph node was seen in only three 160 μ g-dosed rats on Day 91, together with slight or moderate formation of aggregated MWCNT-laden macrophages. The MWCNT deposition and aggregates of MWCNT-laden macrophages were much milder in the parathymic lymph node than in the posterior mediastinal lymph node. The present finding of MWCNT deposition in the LALN suggests that the right and left posterior mediastinal lymph nodes receive MWCNT from the alveolar space and interstitium through a lymphatic drainage pathway, while a small fraction of MWCNT is translocated to the parathymic lymph node through different drainage pathways. Morrow¹⁰⁾ argued that there are two different lymphatic pathways for pulmonary dust clearance, i.e., deep-set and pleural drainage pathways, and that the pleural lymphatics differ topographically from the deep-set in that the pleural pathway follows the surface of the lung segments and lobes to the hilar region. Therefore, it is likely that the intratracheally instilled MWCNT fibers depositing on the alveolar wall would migrate from the alveolar interstitium through the pleural lymphatic pathway into the posterior mediastinal lymph node and to a lesser extent into the parathymic lymph node, and finally into the blood stream at the subclavian vein^{9, 10)}, thereby reaching the reticuloendothelial organs such as spleen and liver.

The LALN such as the posterior mediastinal lymph node, are recognized as a structure important to functioning of the systemic immune system⁸⁾. Notably, Mitchell *et al.*¹¹⁾ reported that the systemic immune function is suppressed by repeated inhalation exposure of male mice to MWCNT aerosol at 0.3–5 mg/m³, and hypothesized that an extrapulmonary mechanism, transforming growth factor- β released from the MWCNT-laden alveolar macrophages in the lung, activates the cyclooxygenase pathway in the spleen, ultimately causing T-cell dysfunction and altered systemic immunity. However, Mitchell *et al.*¹²⁾ argued that the translocation of MWCNT from the lung to the spleen through circulation was unlikely, since no sign of foreign material was detected in the spleen following inhalation exposure. Further studies will be needed to examine whether or not MWCNT-induced nodal lesions varying from the small aggregates of several MWCNT-laden macrophages found in the present study to the reported formation of granulomas and associated inflammation found by Ma-Hock *et al.*¹³⁾, damages the posterior mediastinal lymph node to the extent that the systemic immune function is suppressed.

In conclusion, MWCNT, intratracheally instilled in male F344 rats, was found to migrate to the right and left posterior mediastinal lymph nodes and the parathymic lymph node. The deposition of MWCNT in these lymph nodes gradually dose-dependently increased during the postexposure period, and culminating in the formation of small aggregates of MWCNT-laden macrophages on Day 91, which possibly progress to form microgranulomas. TEM observation confirmed presence of MWCNT fibers in the nodal macrophage, which were characterized by a multi-walled cylindrical structure.

The present study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare of Japan.

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Illustration (picture/graph)

Table 1. Deposition of intratracheally instilled MWCNT and histopathologic changes in LALN at a dose of 0 (vehicle), 40 or 160 μg in the rats sacrificed on different days after instillation

Dose of MWCNT (/rat) Days after instillation	0 μg				40 μg				160 μg			
	1	7	28	91	1	7	28	91	1	7	28	91
Posterior mediastinal lymph node, right												
<No. of rats examined>	<6>	<2>	<8>	<8>	<3>	<4>	<8>	<7>	<5>	<4>	<7>	<8>
MWCNT deposition ¹	0	0	0	0	0	2	5	4	0	4	5	8
(Slight)						(2)	(5)	(2)		(4)	(3)	(0)
(Moderate)								(2)			(2)	(8)
Aggregate of MWCNT-laden macrophages	0	0	0	0	0	0	0	2	0	0	0	6
Posterior mediastinal lymph node, left												
<No. of rats examined>	<7>	<8>	<8>	<8>	<7>	<8>	<8>	<8>	<8>	<8>	<7>	<8>
MWCNT deposition	0	0	0	0	0	1	1	7	0	6	7	7
(Slight)						(1)	(1)	(4)		(6)	(1)	(2)
(Moderate)								(3)			(6)	(5)
Aggregate of MWCNT-laden macrophages	0	0	0	0	0	0	0	2	0	0	0	5
Parathymic lymph node												
<No. of rats examined>	<8>	<7>	<8>	<8>	<8>	<7>	<8>	<8>	<8>	<7>	<8>	<8>
MWCNT deposition	0	0	0	0	0	0	0	0	0	0	0	3
Slight												(1)
Moderate												(2)
Aggregate of MWCNT-laden macrophages	0	0	0	0	0	0	0	0	0	0	0	2

¹MWCNT fibers were engulfed by macrophages.

Because of failure in sampling the right posterior mediastinal lymph node, the right side of several rats was not available for histopathological analysis.

Applicant's summary and conclusion

Conclusions

MWCNT, intratracheally instilled in male F344 rats, was found to migrate to the right and left posterior mediastinal lymph nodes and the parathymic lymph node. The deposition of MWCNT in these lymph nodes gradually dose-dependently increased during the postexposure period, and culminating in the formation of small aggregates of MWCNT-laden macrophages on Day 91, which possibly progress to form microgranulomas. TEM observation confirmed presence of MWCNT fibers in the nodal macrophage, which were characterized by a multi-walled cylindrical structure.

Endpoint study record: Basic toxicokinetics.001 NC7000 Oral**Administrative Data**

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies Result of EU project (Nanogenotox)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Jacobsen et al.		WP 7: Toxicokinetics and tissue distribution of MNs for specification of organs at risk for genotoxicity testing. Deliverable 7: Identification of target organs and biodistribution including ADME parameters.	EU 2nd Health Programme "Nanogenotox"					

Data access

data published

Materials and methods**Type of method**

in vivo

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials**Test material identity**

Identifier	Identity
	Nanocyl NC7000

Radiolabelling

yes

Test animals**Species**

rat

Strain

Wistar

Sex

male/female

Administration / exposure**Route of administration**

oral: gavage

Vehicle

other: 0.05 wt% Rat Serum Albumin

Duration and frequency of treatment / exposure

Single and Repeated for 5 consecutive days

Doses / concentrations

2.56 mg/ml

Dose amount : single administration; 10.9-11.3 mg/kg-bw for male and 10.9-11.3 mg/kg-bw for female

Repeated administration; 48-50 mg/kg-bw for male and 54.5-56.5 mg/kg-bw for female

No. of animals per sex per dose

18 male and 12 female

Control animals

yes, concurrent vehicle

Details on study design

In the frame of the Nanogenotox program, eighteen male (n=18) and 12 female (n=12) rats received a single (day 1) or repeated (on 5 consecutive days, day 1-5) oral administrations of ¹⁴C labelled Nanocyl NC7000 dispersed in 0.05 wt% Rat Serum Albumin (RSA) in ultra pure water. ¹⁴C-labelling of carbon nanotubes was carried out using a three steps chemical process [Georgin et al., 2009]. The single dose groups received a dose per animal between 9.6 – 10 mg/kg b.w. for male animals, and 10.9 – 11.3 mg/kg b.w. for female animals. After single treatment, radioactivity in tissues was evaluated in 3 male or female rats at days 2 and 90. Vehicle treated animals were investigated as follows; males n=2 at days 2, 6, and 90, and females n=1 at days 2, 6 and 90. Various organs and blood were collected including liver, lung, spleen, kidney, heart and testes.

Results and discussions**Main ADME results**

Type
distribution

Bioaccessibility**Any other information on results incl. tables**

After oral administration, ^{14}C labelled MWCNTs didn't show translocation from the GI-tract into the systemic circulation or any of the organs investigated (including spleen, liver, and lung).

Endpoint study record: Basic toxicokinetics.002 NC7000 intravenous**Administrative Data**

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Result of EU project (Nanogenotox)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Jacobsen et al.		WP 7: Toxicokinetics and tissue distribution of MNs for specification of organs at risk for genotoxicity testing. Deliverable 7: Identification of target organs and biodistribution including ADME parameters.	EU 2nd Health Programme "Nanogenotox"					

Data access

data published

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Test materials**Test material identity**

Identifier	Identity
	Nanocyl NC7000

Radiolabelling

yes

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Administration / exposure

Route of administration

intravenous

Vehicle

other: 0.5% Rat Serum Albumin

Duration and frequency of treatment / exposure

single and repeated for 5 consecutive days

Doses / concentrations

2.56 mg/ml

Dose amount : single administration; 10.9-11.3 mg/kg-bw for male and 10.9-11.3 mg/kg-bw for female

Repeated administration; 48-50 mg/kg-bw for male and 54.5-56.5 mg/kg-bw for female

No. of animals per sex per dose

18 male and 12 female

Control animals

yes, concurrent vehicle

Details on study design

In the frame of the Nanogenotox program, eighteen male (n=18) and 12 female (n=12) rats received a single (day 1) or repeated (on 5 consecutive days, day 1-5) intravenous administrations of ¹⁴C labelled Nanocyl NC7000 dispersed in 0.05 wt% Rat Serum Albumin (RSA) in ultra pure water. ¹⁴C-labelling of carbon nanotubes was carried out using a three steps chemical process [Georgin et al., 2009]. The repeated dose groups received a total cumulative dose per animal between 48 – 50 mg/kg b.w after 5 days treatment, for male animals, and 54.5 – 56.5 mg/kg b.w. for female animals. Tissue samples were obtained at various time points after the administration. Repeated treatments tissue levels were evaluated for 3 males at days 6, 14, 30 and 90, and for females at days 6 and 90. Vehicle treated animals were investigated as follows; males n=2 at days 2, 6, and 90, and females n=1 at days 2, 6 and 90. Various organs and blood were collected including liver, lung, spleen, kidney, heart and testes.

Results and discussions

Main ADME results

Type
distribution

Bioaccessibility

Any other information on results incl. tables

After a single intravenous dose of ^{14}C -**Nanocyl NC7000** to male rats most of the injected dose was observed in liver (24%) and lung (25%) at day 1, with a few percent present in spleen, kidneys, heart and testes. At day 90, 20% of injected dose is still observed in organs (cumulated data from all organs analysed), as compared to 65% observed at day 1, with liver and lung being the major targeted organs. After the repeated intravenous dosing for male rats higher organ levels were observed when compared to the single dose. In liver, the accumulated dose is 5-fold higher than the one observed in single injection. However, the percent of total injected dose observed at day 6 in all organs remains in a similar range to that observed by single dosage (ca. 39% instead 65% of the administered dose). Between day 6 and day 90, the percent of total injected dose measured decreased from 39% to 11% in the organs evaluated. As compared to single dose, for which both liver and lung were the main target organs, repeated dosages involved a preferential accumulation in the liver. A decrease of organ loading is observed from day 1 to day 90, except for lung in which a 4% of the injected dose is observed at any time. Overall, a 60% reduction of the total injected dose was observed between day 1 and day 90. The MWCNTs were found to display particular bioaccumulation and biopersistence properties in the various organs evaluated. **Nanocyl NC7000** showed higher bioaccumulation.

Table 6-2A, % of injected dose in organs in rat males (single dose)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
IV NM 400	1,92 ± 0,33	23,79 ± 3,89	24,82 ± 1,76	1,89 ± 0,96	0,00	0,24 ± 0,04	0,00	0,22 ± 0,05		12,5 ± 2,5	65% ± 9%
IV NM 401	0,52 ± 0,42	15,11 ± 6	1,78 ± 1,5	0,37 ± 0,3	0,00	0,00	0,00	0,00	0,04 ± 0,01	0,00	17,8% ± 8%
Graphistrength C100	0,55 ± 0,14	6,98 ± 0,64	0,27 ± 0,13	0,07 ± 0,04	0,00	0,00	0,00	0,00		0,3 ± 0,3	8,2% ± 1,2%
IV NM NRCWE-006	1 ± 0,54	28,21 ± 8,37	1,2 ± 0,65	0,25 ± 0,04	0,00	0,00	0,00	0,16 ± 0,74	0,32 ± 0,19	0,00	31,1% ± 10,5%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
IV NM 400	0,39 ± 0,25	8 ± 1,83	11,4 ± 2,8	0,00	0,00	0,00	0,00	0,00	0,24 ± 0,15	0,00	20% ± 5%
IV NM 401	0,38 ± 0	3,46 ± 0,42	0,59 ± 0,17	0,13 ± 0,1	0,00	0,00	0,00	0,05 ± 0,04	0,09 ± 0,004	0,00	4,7% ± 0,7%
Graphistrength C100	0,23 ± 0,13	3,15 ± 2,2	0,3 ± 0,11	0,00	0,00	0,00	0,00	0,00		0,00	3,7% ± 2,4%
IV NM NRCWE-006	1 ± 0,83	16,40 ± 4,6	1 ± 0,73	0,22 ± 0,06	0,00	0,00	0,00	0,33 ± 0,21	2,12 ± 2	0,00	21% ± 8,4%

Table 6-2B, % of injected dose in organs in rat females (single dose)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	0,69 ± 0,09	24,93 ± 14,6	1,15 ± 0,52	0,59 ± 0,35	0,00	0,00	0,00	0,00		10,8 ± 0,94	38,2% ± 16,6%
IV NM 401	0,22 ± 0,14	6,37 ± 3,87	1,03 ± 0,75	0,28 ± 0,02	0,00	0,00	0,00	0,02 ± 0,02	0,01 ± 0,6	0,00	7,9% ± 5,4%
Graphistrengt C100	0,62 ± 0,33	23 ± 5	0,54 ± 0,19	0,08 ± 0,04	0,00	0,00	0,00	0,00		0,44 ± 0,03	24,6% ± 5,6%
IV NM NRCWE-006	0,85 ± 0,56	35,6 ± 18,2	2,17 ± 1,27	0,26 ± 0,21	0,00	0,00	0,05 ± 0,05	0,00	0,026 ± 0,02	2,73 ± 0,96	42% ± 21%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Ovaries	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	0,36 ± 0,24	3,4 ± 0,61	8,2 ± 0,56	0,00	0,00	0,00	0,00	0,00	0,09 ± 0,04	0,00	12% ± 1,5%
IV NM 401	0,27 ± 0,11	2,5 ± 0,93	0,7 ± 0,28	0,18 ± 0,13	0,00	0,00	0,00	0,00	0,04 ± 0,15	0,00	3,7% ± 1,6%
Graphistrengt C100	0,57 ± 0,14	5,5 ± 1,1	0,54 ± 0,25	0,00	0,00	0,00	0,00	0,00		0,00	6,6% ± 1,5%
IV NM NRCWE-006	1,32 ± 0,63	17,30 ± 7,08	1,83 ± 0,56	0,28 ± 0,17	0,00	0,00	0,00	0,00	0,11 ± 0,23	0,00	20,8% ± 8,7

Table 6-3A: % of injected dose in organs in rat males (repeated doses)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	2,35 ± 0,39	25,42 ± 3,80	4,40 ± 4	1,30 ± 0,63	0,00	0,17 ± 0,10	0,07 ± 0,01	0,27 ± 0,09		5,17 ± 2,1	39% ± 10 %
IV NM 401	0,3 ± 0,04	3,96 ± 1,2	1,05 ± 1	0,10 ± 0,07	0,00	0,00	0,00	0,06 ± 0,03	0,10 ± 0,60	0,00	5,6% ± 3 %
Graphistrengt C100	0,25 ± 0,06	4,78 ± 1,8	0,11 ± 0,03	0,02 ± 0,005	0,00	0,00	0,00	0,00		0,14 ± 0,03	5,3% ± 1,9%
IV NM NRCWE-006	0,71 ± 0,5	22,96 ± 18,8	1,56 ± 1,2	0,51 ± 0,21	0,00	0,07 ± 0,06	0,00	0,00	0,04 ± 0,06	0,25 ± 0,04	26,1% ± 20,5%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Ovaries	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	2,20 ± 1,22	35,21 ± 15,6	4,42 ± 3,26	0,43 ± 0,26	0,00	0,00	0,1 ± 0,04	0,09 ± 0,05		2,12 ± 0,95	44,6% ± 21%
IV NM 401	0,58 ± 0,22	5,82 ± 0,97	0,41 ± 0,14	0,07 ± 0,04	0,00	0,00	0,00	0,00	0,01 ± 0,007	0,00	6,9 % ± 1,5%
Graphistrengt C100	0,30 ± 0,03	4,29 ± 0,4	0,1 ± 0,04	0,01 ± 0,006	0,00	0,00	0,00	0,02 ± 0,01		0,04 ± 0,02	4,7% ± 0,5%
IV NM	1,57 ±	34,5	1,9 ±	0,19 ±	0,00	0,00	0,00	0,00	0,22 ±	0,00	38,4% ±

NRCWE-006	0,26	± 5	0,48	0,03					0,01		5,8%
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Table 6-3B,% of injected dose in organs in rat females (repeated doses)

24h	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Testes	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	2,26 ± 0,6	34,94 ± 16,02	32,78 ± 11,21	1,30 ± 0,46	0,00	0,28 ± 0,09	0,07 ± 0,02	0,02 ± 0,004	5,87 ± 0,40		77,3% ± 28,8%
IV NM 401	0,13 ± 0,1	4,1 ± 0,77	0,55 ± 0,026	0,09 ± 0,07	0,00	0,03 ± 0,02	0,00	0,006 ± 0,003	0,00	0,005 ± 0,002	5% ± 0,9%
Graphistrengt C100	0,42 ± 0,19	10,59 ± 5	0,40 ± 0,4	0,04 ± 0,02	0,00	0,00	0,00	0,00	0,16 ± 0,30		11,6% ± 5,9 %
IV NM NRCWE-006	0,76 ± 0,4	34,76 ± 20,1	2,42 ± 2	0,19 ± 0,15	0,00	0,06 ± 0,03	0,00	0,01 ± 0,004	0,30 ± 0,09	0,08 ± 0,03	38,6% ± 22,7%
Day 90	Spleen	Liver	Lung	Kidney	Brain	Heart	Thymus	Ovaries	Pancreatic lymph node	Blood (total body)	TOTAL
NC 7000	0,21 ± 0,13	7,92 ± 2,46	4,68 ± 0,97	0,04 ± 0,01	0,00	0,00	0,00	0,00	0,00	0,12 ± 0,06	13% ± 3,6%
IV NM 401	0,33 ± 0,05	2,7 ± 0,39	0,39 ± 0,06	0,05 ± 0,01	0,00	0,00	0,00	0,00	0,00	0,1 ± 0,1	3,6% ± 0,6%
Graphistrengt C100	0,41 ± 0,02	3,21 ± 2,79	0,18 ± 0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00	3,8% ± 2,9%
IV NM NRCWE-006	1,82 ± 0,55	22,02 ± 6,88	2,28 ± 0,95	0,31 ± 0,11	0,00	0,00	0,00	0,00	0,00	0,58 ± 0,34	27% ± 8,8%

Endpoint study record: Nikkiso MWCNT: Basic toxicokinetics:001**Administrative Data**

Purpose flag	key 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	Oyabu T, Myojo T, Morimoto Y, Ogami A, Hirohashi M, Yamamoto M, Todoroki M, Mizuguchi Y, Hashiba M, Lee BW, Shimada M, Wang WN, Uchida K, Endoh S, Kobayashi N, Yamamoto K, Fujita K, Mizuno K, Inada M, Nakazato T, Nakanishi J, Tanaka I.	2011	Biopersistence of inhaled MWCNT in rat lungs in a 4-week well-characterized exposure	Inhalation Toxicology, 23 (13), 784-791 (2011)					

Data access

data published

Materials and methods**Type of method**

in vivo

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:	Nikkiso MWCNT

Radiolabelling

no

Test animals

Species

rat

Strain

Wistar

Sex

male

Administration / exposure

Route of administration

inhalation: aerosol

Details on exposure

TYPE OF INHALATION EXPOSURE:

whole body

GENERATION OF TEST ATMOSPHERE / CHAMPER DESCRIPTION

- Exposure apparatus: Exposure chamber connected to pressureized nebulizer
- Method of holding animals in test chamber: whole body inhalation
- System of generating particulates/aerosols: MWCNT in the aqueous solution of 0.5 mg/ml Triton X-100 was atomized by a pressurized nebulizer and introduced in the exposure chamber after being dried by a mist dryer.
- Method of particle size determination: The size distribution of the MWCNT aerosol and Triton X-100 aerosol particles at the exit of the nebulizer and inside the exposure chamber were measured repeatedly using a particle spectrometer consisting of a differential mobility analyzer and a condensation particle counter.

TEST ATMOSPHERE (if not tabulated) GSD (Geometric st. dev.):

63 nm

Duration and frequency of treatment / exposure

6h/d x 5d/w x 4w

Doses / concentrations

0.37 +/- 0.18 mg/m³

No. of animals per sex per dose

30 rats per group. Three groups (MWCNT, Triton X-100 (negative control) and unexposed) were set.

Control animals

yes, concurrent no treatment

Details on dosing and sampling

After the completion of inhalation for 4 weeks, 10 rats from each group were dissected at 3 days, 1 month and 3 months. The lungs were isolated. The amounts of MWCNT deposited in the rat lungs were determined by the X-ray diffraction method (XRD) and elemental carbon analysis (ECA).

Results and discussions**Main ADME results**

Type	
distribution	The pulmonary deposition fraction was calculated as 18% (XRD) or 20% (ECA) of inhaled MWCNT
excretion	Biological half-life time eliminated from the rat lung was calculated as 51 or 54 days.

Pharmacokinetic studies**Details on distribution in tissues**

The average deposited amounts at 3 days after inhalation were 68 µg/lung by XRD method and 76 µg/lung by elemental carbon analysis. The calculated deposition fractions were 18% and 20% in each analysis.

Details on excretion

The amount of retained MWCNT in the lungs until 3 months after inhalation decreased exponentially and the calculated biological half times of MWCNT were 51 days and 54 days, respectively.

Overall remarks, attachments**Attached full study report**

Attached full study report
④ MWCNT (Inhal.Toxcol.P784-791).pdf / 705.09 KB (application/pdf)

Illustration (picture/graph)

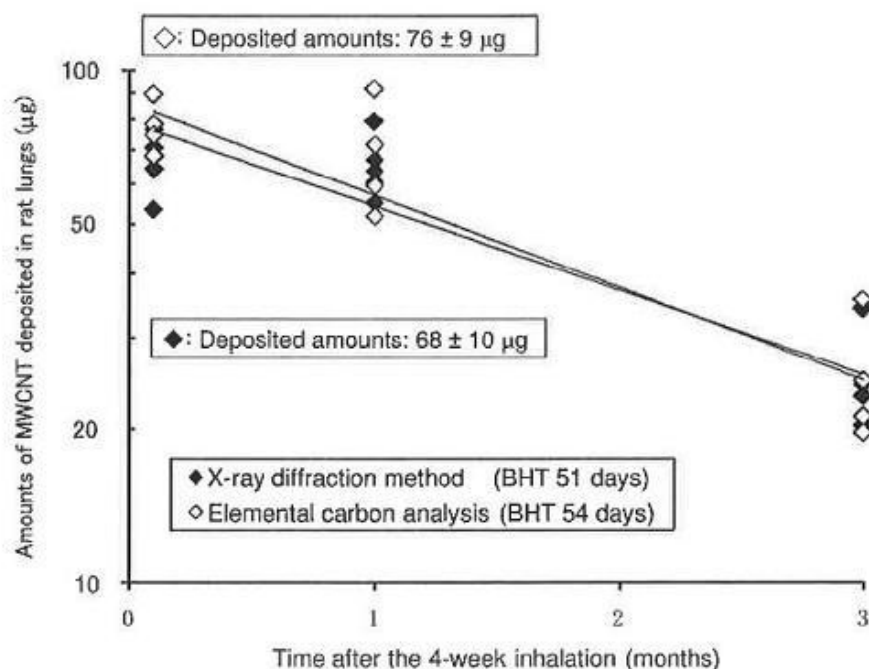


Figure 5. Temporal change of MWCNT in rat lungs after 4 weeks inhalation.

Applicant's summary and conclusion

Conclusions

The clearance of MWCNT in the lungs was not delayed and the significant effects which inhibit the clearance were not observed. Thus the obvious hazard potential of MWCNT was not observed in this experimental condition.

7.1.2 Dermal absorption

7.2 Acute Toxicity

7.2.1 Acute toxicity: oral

Endpoint study record: Baytube: Acute toxicity: oral.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
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 MaterialScience	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 423 (Acute Oral toxicity - Acute Toxic Class Method)	

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Test animals**Species**

rat

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
	LD50	> 5000 mg/kg bw			

Endpoint study record: Graphistrength C100: Acute toxicity: oral.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 423)

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**Test type**

standard acute method

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	

GLP compliance

yes

Test materials**Test material identity**

Identifier	Identity
	Arkema Graphistrength C100

Details on test material

- Name of test material (as cited in study report): Graphistrength C100
- Other: Micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability

Test animals**Species**

rat

Strain

Sprague-Dawley

Sex

female

Administration / exposure**Route of administration**

oral: gavage

Vehicle

other: 0.5% methylcellulose

Doses

300, 2000 mg/kg

No. of animals per sex per dose

3 females/dose/group

Control animals

no

Details on study design

The acute oral toxicity of Graphistrength C100 (micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability) was evaluated in rats according to OECD TG423 and Good Laboratory Practice Regulations. The test item was prepared in 0.5% methylcellulose and was administered by oral route (gavage), to groups of three fasted females Sprague-Dawley rats. Clinical signs, mortality and body weight gain were checked for a period of up to 14 days following the single administration of the test item. All animals were subjected to necropsy.

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD0	>= 2000 mg/kg bw			

Mortality

No deaths were observed.

Clinical signs

Hypoactivity, piloerection and dyspnea were noted in 3/6 animals on day 1, at 2000 mg/Kg.

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

No deaths and no clinical signs were noted at 300 mg/Kg. No deaths occurred but hypoactivity, piloerection and dyspnea were noted in 3/6 animals on day 1, at 2000 mg/Kg. The oral LD0 of Graphistrength C100 (micronized) was equal to or higher than 2000 mg/Kg in rats.

Endpoint study record: Nanocyl NC7000: Acute toxicity: oral.001**Administrative Data****Purpose flag** supporting study**Study result type** experimental result**Reliability** 2 (reliable with restrictions)**Rationale for reliability incl. Study meets the requirements of research purposes but is not suitable for deficiencies** regulatory hazard assessment**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**Test type**

Fixed dose procedure

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 420 (Acute Oral Toxicity - Fixed Dose Method)	yes (Highest doses impossible to reach due to the physico-chemical)

GLP compliance

no

Test materials**Test material identity**

Identifier	Identity
	Nanocyl NC7000

Test animals**Species**

rat

Strain

Sprague-Dawley

Sex

female

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

- Source: Ico: OFA-SD (IOPS Caw), Charles River
- Age at study initiation: 12 weeks
- Weight at study initiation: 230g
- Fasting period before study: No
- Housing:
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: one week

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20-22°C
- Humidity (%): 45-65%
- Air changes (per hr):
- Photoperiod (hrs dark / hrs light): 12 hours

Administration / exposure**Route of administration**

oral: gavage

Vehicle

other: hydroxypropyl cellulose (HPC)

Details on oral exposure**VEHICLE**

- Concentration in vehicle: 1% p/v
- Amount of vehicle (if gavage): 5ml/kg
- Justification for choice of vehicle: The hydroxypropyl cellulose (HPC) or Klucel is a non ionic cellulosic polymer. This thickener-viscosifier agent has been chosen on the basis of an in vivo study on mouse about the toxicological effects of copper nanoparticles where the suspension agent was the hydroxypropylmethyl cellulose (HPMC) (1%) and on the other end, on the basis of tests realised previously with other agents (tween, SDS, gum arabic...) The conclusions of this study on suspension was that HPC was the best dispersion agent. Moreover, this agent is used as excipient in numerous pharmaceutical preparations.

MAXIMUM DOSE VOLUME APPLIED: 5ml/kg

DOSAGE PREPARATION (if unusual):

CNT are dispersed in HPC (1% p/v) at concentrations of 0.1mg/ml to 20mg/ml. The suspensions are alternatively "vortexed" during 3 minutes and are subjected to ultrasounds in a bath (Branson 2510) during 10 minutes and this during 1 hour.

Doses

0.5mg/kg ; 1mg/kg ; 5mg/kg ; 50mg/kg ; 100mg/kg

No. of animals per sex per dose

4

Control animals

yes

Details on study design

- Duration of observation period following administration: 1 day
- Frequency of observations and weighing: The animals are observed 10, 30 minutes, 18 and 24 hours after administration
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, organ weights, blood analysis, macroscopic examinations of organs and histopathology, other:
- After 24 hours, the animals are anaesthetized (nembutal, 60 mg/kg. An incision is done from the cloaca to the chin following the medio-ventral line. The inferior vena cava is isolated in order to take blood in this vessel. Plasma is obtained after centrifugation.
- Measurements of: sodium, potassium, glucose, total cholesterol, alkaline phosphatase and albumin.
- Measurements of biochemical parameters reflecting the hepatic function such as the aspartate aminotransferase (TGO) and alanine aminotransferase (TGP) and of biochemical parameters reflecting the renal function such as urea and creatinine.
- Macroscopic examination and autopsy: organs observed: stomach, small and large intestine, liver, pancreas, kidneys, spleen, the heart-lung block, the reproductive organs and the bladder.
- Histological analysis: observed organs: stomach, small and large intestine, liver, pancreas, kidneys, spleen.

The samples are wrapped up in paraffin blocks which are cut in slices of 6µm thick. The slices are put on microscop plates which are developed by a technique of conventional histology coloration, the hematoxylin and eosine (H&E). The slides are observed by optical microscopy (Olympus Provis AX70) and pictures are taken with a camera (Zeiss Axiocam).

The histological cuts have been done in the stomach, small and large intestine, spleen, pancreas, liver and kidneys. The other organs (the heart-lung machine, the reproductive apparatus and the bladder) are stored in formalin (10%) if further investigations are needed. The histopathological examination has been realised in collaboration with the Institute of Pathology and Genetic (IPG, Gosselies).

Statistics

- Statistical studies between tests groups and control group have been realised for each studied biochemical parameter using a non parametric test Mann Whitney (GraphPad Prism software).

Results and discussions

Effect levels

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	other: iver toxicity	> 100 mg/kg bw			No dose dependent increase of granulomatous changes in the liver 24 hours after a single dosage (0.5, 1, 5, 50, 100 mg/kg).

Mortality

No mortality

Clinical signs

No particular symptom

Gross pathology

no morphological changes

Other findings

- Biochemical parameters in blood plasma: results are in table 1. All the values of the measured parameters are within the normal values described for the rat. The absence of significant increase of plasmatic enzymes TGO and TGP suggests that the hepatic function is not affected. The absence of increase of the concentration of urea and plasmatic creatinine suggests that the renal function is not affected either by the administration of CNT. If significant differences ($p < 0.005$) are observed in group 2, 3, 4 and 5 compared with the control group for total proteins, the values are staying within the authorised values described for the rats. It is the same for group 4 in relation to the control group for creatinine.

- Organ weights: The liver, the kidneys and the spleen have been weighted and the weight of the organs of the exposed groups were similar to the one of the control group. (table 2)

- Gross examination and necropsy: No changes have been observed at the level of the removed organs of the gastro-intestinal tract such as the stomach, small and large intestine but also incidental glandular organs such as liver and pancreas.

- Histopathology: the histopathological examination of stomach, colon and small intestine did not reveal signs of toxicity. It is the same for the spleen, the pancreas and the kidneys. Small inflammatory granulomatous changes composed mainly of polynuclear and lymphocytes are observed in the interlobular space in the liver (Kiernan's space) and also in the hepatic parenchyma and this from the dose of 0.5mg/kg. The presence of those granulomatous changes is localised and their size is very limited. Beside the presence of those granulomatous changes, the cellular structure is well preserved and no steatosis is observed, nor cholestasis, hepatocellular insufficiency or sign of suffering. The set of hepatic granulomatous changes of each rat exposed to the suspension of nanoparticles have been quantified. Considering that the size of the slides of liver is homogeneous, the number of granulomatous changes present on each slide have been counted (minimum 3-5 sections by liver of animals) in order to determined the mean for minimum 3 sections. The surfaces of the slides of liver were quantified with the help of a software of morphometry (Histolab) which allows to digitalised surfaces in order to determined the number of granulomatous changes or the quantified surface for a granulomatous change. The mean surface of the liver evaluated for the counts of granulomatous changes is $1026 \pm 100 \text{ mm}^2$. (Table 3). There is no dose dependent difference between the groups that have received carbon nanotubes and the control group.

Any other information on results incl. tables

Table 1: Biochemical parameters in blood plasma (mean $\pm$ S.D. (n=4, * : $p<0.05$ compared with the control group))

Parameters (n. physiol.)	Control HPC	Group 1 ; 0.5 mg/kg	Group 2 ; 1 mg/kg	Group 3 ; 5 mg/kg	Group 4 ; 50 mg/kg	Group 5 ; 100 mg/kg
Sodium 130-150 (mmol/L)	145.30 $\pm$ 1.50	146.50 $\pm$ 1.0	145.50 $\pm$ 1.29	145.50 $\pm$ 1.00	147.50 $\pm$ 8.50	150.80 $\pm$ 7.41
Potassium 4.5-6.0 (mmol/L)	3.75 $\pm$ 0.42	4.07 $\pm$ 1.15	3.77 $\pm$ 0.23	3.25 $\pm$ 0.42	3.52 $\pm$ 0.38	4.35 $\pm$ 1.45
Glucose 0.89-1.833 (g/L)	2.14 $\pm$ 0.15	2.14 $\pm$ 0.22	2.34 $\pm$ 0.12	2.19 $\pm$ 0.26	2.25 $\pm$ 0.31	2.31 $\pm$ 0.63
Urea 0.32-0.54 (g/L)	0.26 $\pm$ 0.018	0.31 $\pm$ 0.07	0.28 $\pm$ 0.03	0.29 $\pm$ 0.05	0.27 $\pm$ 0.03	0.31 $\pm$ 0.02
Creatinine 3.9-22.9 (mg/L)	4.37 $\pm$ 0.71	3.37 $\pm$ 0.25	3.60 $\pm$ 0.33	3.17 $\pm$ 0.51	3.40 $\pm$ 0.44	3.45 $\pm$ 0.37
Total proteins 59.0-84.0 (g/L)	61.75 $\pm$ 1.71	58.75 $\pm$ 2.99	55.50 $\pm$ 1.73*	53.75 $\pm$ 1.26*	51.50 $\pm$ 2.38*	54.00 $\pm$ 3.74*
Albumine 32.0-43.0 (g/L)	39.25 $\pm$ 4.27	37.25 $\pm$ 3.86	35.00 $\pm$ 1.41	34.50 $\pm$ 0.58	29.25 $\pm$ 2.22	28.75 $\pm$ 9.29
PA 39-216 (UI/L)	112.00 $\pm$ 20.41	117.00 $\pm$ 16.25	142.30 $\pm$ 20.17	138.50 $\pm$ 13.10	134.00 $\pm$ 16.08	129.30 $\pm$ 15.37
TGO 39-92 (UI/L)	100.30 $\pm$ 26.00	78.75 $\pm$ 18.03	94.75 $\pm$ 15.92	83.75 $\pm$ 7.63	58.75 $\pm$ 9.21	73.75 $\pm$ 10.44
TGP 17-50 (UI/L)	34.75 $\pm$ 8.99	33.25 $\pm$ 3.30	34.00 $\pm$ 3.16	39.25 $\pm$ 3.30	31.50 $\pm$ 3.87	33.25 $\pm$ 3.50
Total cholesterol 0.5-1.0 (g/L)	0.70 $\pm$ 0.05	0.68 $\pm$ 0.11	0.68 $\pm$ 0.12	0.70 $\pm$ 0.07	0.64 $\pm$ 0.15	0.64 $\pm$ 0.07

Table 2: Weights (g) of various target organs: spleen, liver, kidneys: (mean $\pm$ S.D.)

	spleen	liver	left kidney	right kidney
Control	0.50 $\pm$ 0.10	7.46 $\pm$ 0.40	0.60 $\pm$ 0.05	0.7 $\pm$ 0.00
0.5 mg/kg	0.50 $\pm$ 0.09	6.90 $\pm$ 0.55	0.67 $\pm$ 0.04	0.67 $\pm$ 0.05
1 mg/kg	0.65 $\pm$ 0.05	7.90 $\pm$ 0.69	0.65 $\pm$ 0.05	0.67 $\pm$ 0.05
5 mg/kg	0.50 $\pm$ 0.04	8.05 $\pm$ 0.42	0.60 $\pm$ 0.00	0.65 $\pm$ 0.06
50 mg/kg	0.57 $\pm$ 0.05	8.27 $\pm$ 0.37	0.60 $\pm$ 0.00	0.67 $\pm$ 0.05
100 mg/kg	0.60 $\pm$ 0.05	7.45 $\pm$ 0.69	0.62 $\pm$ 0.05	0.62 $\pm$ 0.09

Table 3: Quantification of the surface of the liver for one granuloma (mm²) and the number of granuloma per cm² (mean $\pm$ SEM) (* = $p < 0.005$; ** = $p < 0.001$ compared with the control):

	1 granuloma / X mm²	granuloma / cm²
Control	141 $\pm$ 60	<1
0.5 mg/kg	22 $\pm$ 9*	5 $\pm$ 3
1 mg/kg	19 $\pm$ 10**	7 $\pm$ 4
5 mg/kg	25 $\pm$ 14*	5 $\pm$ 2
50 mg/kg	42 $\pm$ 20	2 $\pm$ 2
100 mg/kg	37 $\pm$ 29*	4 $\pm$ 2

Applicant's summary and conclusion

Executive summary

All the animals of the study have survived to the oral administration of CNT and didn't show particular symptoms within 24 hours following the administration of doses from 0.5mg/kg to 100 mg/kg. The analysis of all the biochemical parameters at the plasmatic level did not show abnormal values; all the values of the various measured parameters were within the normal range described for the rat. The gross examination of the viscera did not show particular morphological changes. The liver, the kidneys and the spleen of each animal of the study have been weighted and the weight of the organs of the exposed groups were similar to those of the control group. If no sign of toxicity has been observed for the organs such as the stomach, the colon, the small intestine, the spleen, the pancreas and the kidneys, small inflammatory granulomatous changes are observed in the interlobular space in the liver as well as in the hepatic parenchyma of all treated animals (≥ 0.5 mg/kg). The presence of those granulomatous changes is well localised and their size is limited. Besides the presence of those granulomatous changes, the cellular structure is well preserved and no steatosis is observed, nor cholestasis, hepatocellular insufficiency or another sign of impact. Those granulomatous changes are not dose dependent pronounced. They are more numerous in the groups having received doses of 0.5 to 5 mg/kg. In conclusion, we observed the presence of granulomatous changes in the liver 24 hours after a single exposure to test substance meeting the form described in section 4.5 of the IUCLID dossier. However, the absence of dose dependence questioned the toxicological relevance of this observation.

Endpoint study record: Nikkiso MWCNT: Acute toxicity: oral.001

Administrative Data

Purpose flag	key study
Study result type	experimental result Study period Oct, 2010 to Feb. 2011
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Under GLP and according to OECD TG423

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Masao Sunaga	2011	Acute Oral Toxicity Test of Carbon Nanotube in Rats		Safety Research Institute for Chemical Compounds Co., Ltd.	SR10105			2011-02-27
publication	M. Matsumoto et al. (National Institute of Health Sciences)	2012	No toxicological effects on acute and repeated oral gavage doses of single-wall or multi-wall carbon nanotube in rats	J. Toxicol. Sci. Vol.37, No.3, 346-474, 2012	Safety Research Institute for Chemical Compounds Co., Ltd.				

Materials and methods**Test type**

acute toxic class method

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	

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
	Nikkiso MWCNT

Details on test material

- Name of test material (as cited in study report): Multi-walled carbon nanotube
- Analytical purity: >98%
- Impurities (identity and concentrations): Fe (ca. 20 - 50 ppm); other impurities: unknown
- Lot No.: 04-12/10#1-(4)

- Stability under test conditions: No data.
- Storage condition of test material: room temperature (measured: 21 - 25 °C)

Dose preparation:

The test material was accurately weighed and added with the vehicle to obtain the prescribed concentration and mixed. The mixture was filled up to the prescribed volume and homogenized using an ultrasonic homogenizer (VC-130, Sonics & Materials Inc.) for 5 minutes, and then using a compact ultrasonic cleaning bath (USC-6, Iwaki Glass Co., Ltd.) for 10 minutes. The mixture was homogenized with the ultrasonic homogenizer for 5 minutes, which was used as the test solution. In order not to prevent homogenization, a stirrer was not used.

Test animals

Species

rat

Strain

other: Crl:CD(SD)

Sex

female

Details on test animals and environmental conditions

- Source: Charles River Laboratories Japan, Inc.
- Age at study initiation: 9 weeks old
- Weight at study initiation: female (200 - 207 g)
- Fasting period before study: 17 - 18 hr
- Housing: Animals were individually housed in metallic cages with wire mesh bottoms (260W × 380D × 180H mm)
- Diet: CRF-1; Oriental Yeast Co., Ltd., Japan (ad libitum)
- Water: Tap water (ad libitum)
- Acclimation period: 5 days for 1st group and 8 days for 2nd group

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22 ± 3 °C (measured: 20 - 23 °C)
- Humidity (%): 50 ± 20 % (measured: 40 - 51%)
- Air changes (per hr): 10-15 time/hour
- Photoperiod (hrs dark / hrs light): 12 hr dark/12 hr light

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: 5% Acacia aqueous solution

Details on oral exposure**MAXIMUM DOSE VOLUME APPLIED:**

20 ml/kg b.w., 4 times, 1 hour interval

Rationale for vehicle selection: In the investigation of the vehicles including gum acacia, carmellose sodium, and corn oil, which are commonly used in toxicity studies, the test material showed the best dispersion state in 5% gum acacia.

Rationale for dose selection: In the assay of dispersion state of the test material, the dose of 2.5 mg/mL was confirmed to be optimum and limit concentration. Ethically, a dosing volume of 20 mL/kg is the limit volume at one time by the oral route. When the dosing volume was dosed several times a day, 4 times seemed to be the limit number of dosing times. Consequently, 4-times dosing at 2.5 mg/mL and 20 mL/kg, starting from a dose of 200 mg/kg, was adopted for the present study. In the 1st group, no death was found in 2 days observation after dosing, and no adverse effects such as a decrease in body weight were noted in the body weights on day 1 after dosing. Therefore, the dose of 200 mg/kg was set for the 2nd group.

Doses

200 mg/kg bw (1st group) and 200 mg/kg bw (2nd group)

No. of animals per sex per dose

3 females/ dose/group

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days

- Frequency of observations and weighing:

OBSERVATION:

Clinical observation was performed consecutively for 1 hour and 2 and 4 hours after last administration on the dosage day and performed twice a day from the next day.

WEIGHING:

Animals were weighed just prior to administration, and 1, 3, 5, 7, 10 and 14 days after administration.

- Necropsy of survivors performed: yes

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD50	> 200 mg/kg bw			

Mortality

No deaths were observed.

Clinical signs

Black feces revealed 1 or 2 days at 200 mg/kg bw after administration.

Body weight

No effects were observed

Gross pathology

No effects were noted. In one animal in the 2nd group (200 mg/kg), dilatation of the renal pelvis in the right and left kidneys was noted. Dilatation of the renal pelvis is a spontaneous change, and accompanied no organic changes in the kidneys; therefore, this change was not considered related to the test material administration.

Any other information on results incl. tables

Table 1 Mortality of female rats in an acute oral toxicity test of MWCNT

	Number of dead animals									
	1 hour after each administration				Hours after the 4 th administration				Days after the administration	Mortality
Group	Pre	1st	2nd	3rd	0.5	1	2	4	1-14	
200 mg/kg	0	0	0	0	0	0	0	0	0	0 / 6

Pre: Pre-administration.

Mortality: Number of dead animals / number of animals dosed

Table 2 General appearance of female rats in an acute oral toxicity test of MWCNT

Group	Findings	1 hour after each administration				Hours after the 4 th administration				Days after the administration		
		Pre	1st	2nd	3rd	0.5	1	2	4	1	2	3-14
200 mg/kg	Number of animals examined	6	6	6	6	6	6	6	6	6	6	6
	No abnormal findings	6	6	6	6	6	6	6	6	0	5	6
	Black feces	0	0	0	0	0	0	0	0	6	1	0

Pre : Pre-administration.

Values are number of animals with findings.

Table 3 Body weight of female rats in an acute oral toxicity test of MWCNT

Group	Body weight (g) on days after the administration							Body weight gain	
	0	1	3	5	7	10	14	g	%
	6 ^{a)}	6	6	6	6	6	6	6	6
200 mg/kg	204 ± 2.6	221 ± 5.8	230.7 ± 3.3	237 ± 10.1	239.7 ± 10	247.2 ± 11.2	251 ± 16.1	47 ± 16.9	23.078 ± 8.462

Day 0 : Pre-administration. a): Number of animals examined

Table 4 Gross findings of female rats in an acute oral toxicity test of MWCNT

Group (Dose) 200 mg/kg	
Number of animals examined	6
No abnormal findings	5
Organ : Findings	1
Kidney : Dilatation, renal pelvis, bilateral	

Values are number of animals with findings.

Overall remarks, attachments

Remarks on results including tables and figures

No indicative of abnormality is observed by twice dose of 200 mg/kg/day.

Attached full study report

Attached full study report
CNT oral dose.pdf / 900.97 KB (application/pdf): Appendix 4

Applicant's summary and conclusion**Conclusions**

The LD₅₀ value of this substance is considered as >200 mg/kg.

Executive summary

No deaths occurred in 200 mg/kg and thus the oral LD₅₀ value in rats for Multi-walled Carbon Nanotube was greater than 200 mg/kg bw in females. Multi-walled Carbon Nanotube administered orally caused no toxicological effects at a dose of 200 mg/kg bw in rats.

Endpoint study record: Nikkiso MWCNT: Acute toxicity: oral.002**Administrative Data**

Purpose flag supporting study
Study result type experimental result **Study period** Sept.2010 - Dec. 2010
Reliability 2 (reliable with restrictions)
Rationale for reliability incl. deficiencies OECD Test Guideline study

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2010	Micronucleus assay of Multiwalled carbon Nanotube B (CNT-B) using mice		Ina research	ZT10127			2010-12-09

Materials and methods**Test type**

other: micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
no guideline followed	other guideline: 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 MWCNT

Details on test material

- Name of test material (as cited in study report): Multi-walled Carbon Nanotubes B (CNT-B)
- Stability: unknown
- Store condition: light shielding, room temperature (accepted range: 1-30°C)
- Storage: test substance storage room

Test animals**Species**

mouse

Strain

other: Crlj:CD1(ICR)

Sex

male

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

- Source: Charles River Laboratories, Japan Inc.
- Age at study initiation: 7 weeks
- Body weight at starting of dosing: 34.5-35.0g (male), 24.3-25.5g (female) in preliminary test, 34.1-38.8g (male) in main test.
- Assigned to test groups randomly: yes
- Fasting period before study: No
- Housing: Animals were individually housed in polycarbonate cage (175W × 245D × 125H mm) with bedding for laboratory animals.
- Diet: CRF-1, Oriental Yeast Co., Ltd., Japan (ad libitum)
- Water: Tap water (ad libitum)- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 21.0-25.0 (measured: 22.7-24.1)
- Humidity (%): 40.0-70.0 (measured: 50.5-64.3)
- Air changes (per hr): 14-19 times
- Lightening hours: 12hrs dark/12hrs light)

Administration / exposure

Route of administration

oral: gavage

Vehicle

CMC (carboxymethyl cellulose)

Details on oral exposure

- Vehicle(s)/solvent(s) used: 0.3%CMC (sodium carboxymethyl cellulose)
- CNT-B suspension of 1mg/ml (maximum concentration) was ultra-sonicated for 5 minutes before use and then diluted with 0.3%CMC to make the concentrations of 0.5 and 0.25 mg/ml for the main test.
- Administration volume: 20mL/kg/day

Doses

5, 10 and 20 mg/kg/day, two times in the interval of 24 hours

No. of animals per sex per dose

6 males/dose

Control animals

yes

Details on study design

The study was conducted in the course of TG 474 experiment. The test was done using 6 male Crlj: CD1(ICR) mice of nine weeks of age in each dose group. The CNTs, which were suspended in the water for injection and is diluted with 0.3% CMC-Na solution, is orally administered two times in the interval of 24 hours at the dose of 5, 10 and 20 mg/kg/day. The observation is carried out after 24 hours of final dose.

Results and discussions

Preliminary study (if fixed dose study)

No indicative of abnormality is observed in each group.

Mortality

No death was observed in all groups.

Clinical signs

No effect was observed.

Body weight

No effect was observed.

Any other information on results incl. tables**Table 1 Clinical observation of mice (TG474)**

Sex	Compound	Dose (mg/kg)	Day 1 of dosing			Day 2 of dosing	
			IB	IA	1h	IB	IA
Male	0.3%CMC ^{a)}	0	N	N	N	N	N
	CNT-B	5	N	N	N	N	N
	CNT-B	10	N	N	N	N	N
	CNT-B	20	N	N	N	N	N
	MMC ^{b)}	5	N	N	N	N	N

IB: Immediately before dose, IA: Immediately after dose, 1h: 1hour post-dose

N: No abnormality

a) Negative control, 0.3% Sodium carboxymethylcellulose solution

b) Positive control, Mytomycin C

Table 2 Body weight of mice (Primary test of TG474)

Sex	Compound	Dose (mg/kg)	Day 1 of dosing ^{a)}	Day 2 of dosing ^{a)}	Day of specimen preparation
Male	0.3%CMC	0	36.4 ± 1.3	37.2 ± 1.5	37.1 ± 1.7
	CNT-B	5	36.3 ± 1.7	36.9 ± 1.5	36.9 ± 1.6
	CNT-B	10	36.3 ± 1.4	36.7 ± 1.3	36.7 ± 1.3
	CNT-B	20	35.9 ± 1.5	36.6 ± 1.5	36.2 ± 1.6
	MMC	5	36.2 ± 1.4	36.7 ± 1.6	36.3 ± 1.5

a): pre-dosing

Data were not statically analyzed

Endpoint study record: Hanwha CM-100: Acute toxicity: oral.001**Administrative Data****Purpose flag**

key study

Study result type

experimental result

Study period

2011

Reliability

2 (reliable with restrictions)

Rationale for reliability deficiencies

incl. Secondary source, but well documented and reliable guideline study.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
secondary source	Ministry of Knowledge and Economy(MKE), Korea	2011	Acute oral toxicity study of MWCNT in rats	Ministry of Knowledge and Economy(MKE), Korea, 2011.Acute oral toxicity study of MWCNT in rats, tested by KCL(Korea conformity Laboratory) (cited in Multi-walled Carbon Nanotubes (MWCNTs))	KCL(Korea conformity Laboratory)				

Materials and methods**Test type**

acute toxic class method

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	

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:	Hanhwa CM-100

Details on test material

- Name of test material (as cited in study report): Multi-Walled Carbon Nanotubes (MWCNTs)
- Analytical purity: ca. 95% based on thermogravimetric analysis
- Other:Identity (purity): Diameter 10~15 nm, length ~200 micron

Remarks:

Manufactured by Hanwha Nanotech Co., Ltd. (Korea)

Test animals**Species**

rat

Strain

Sprague-Dawley

Sex

female

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

- Age at study initiation: 8-9 week old

Administration / exposure**Route of administration**

oral: gavage

Vehicle

other: DPPC solution(5.5mM D-(+)-glucose + 0.6mg/ml Bovine serum albumin + 0.01 mg/kg DPPC

Details on oral exposure

The test substances were dispersed in vehicle at maximum 0.5% therefore the volume of 2,000 mg/kg dose was 400 mL/kg. So the higher dose test (2,000 mg/kg) was not conducted because it is impossible to administer orally.

Vehicle: DPPC solution(5.5mM D-(+)-glucose + 0.6mg/ml Bovine serum albumin + 0.01 mg/kg DPPC in DPBS).

Doses per time period: 2 hr. (Administered three times)

Volume administered or concentration: 20 mL/kg body weight

Doses

300 mg/kg bw

No. of animals per sex per dose

Six

Details on study design

- Duration of observation period following administration: 14 days

- Frequency of observations and weighing: No data

- Necropsy of survivors performed: yes

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD50	> 300 mg/kg bw			

Mortality

Mortality was not observed.

Clinical signs

Clinical sign was not observed.

Gross pathology

In one animal 2nd step (300 mg/kg), there were black substances retention around lung and thymus and adhesion with esophagus.

Applicant's summary and conclusion**Conclusions**

Single, oral administration of MWCNT did not cause any signs of toxicity up to 300 mg/kg body weight.

7.2.2 Acute toxicity: inhalation**Endpoint study record: Baytubes: Acute toxicity: inhalation.001****Administrative Data**

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability deficiencies	incl. Secondary source, but well documented and reliable guideline study.

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
according to	OECD Guideline 403 (Acute Inhalation Toxicity)	

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

Details on test material

The MMAD for CNTs was in the range of 2-3 μm . Rigorously dispersed CNTs (diameter 10-16 nm) had a mean length of 1024 ± 628 nm and 309 ± 334 nm before and after micronization. The surface area of CNT was 253 m²/g.

Test animals**Species**

rat

Strain

Wistar

Administration / exposure**Route of administration**

inhalation

Type of inhalation exposure

nose only

Duration of exposure

ca. 6 h

Concentrations11 and 241 mg Baytubes/m³

reference substances are:

- crystalline quartz (= positive control): 0, 250 mg/m³ (target concentration)
- crystalline quartz (0 positive control): 2247.5 mg/m³ (gravimetrical concentration)
- carbon black (= negative control): 0, 250 mg/m³ (target concentration)
- carbon black (= negative control): 228.6 mg/m³ (gravimetrical concentration)

Any other information on materials and methods incl. tables

Bayer MS (BIAC) performed an acute inhalation toxicity study: Wistar rats were nose-only exposed by inhalation (1x 6h) to 11 and 241 mg Baytubes/m³ and to 248 mg/m³ crystalline quartz (DQ12). The MMAD for CNTs was in the range of 2-3 μm . Inflammatory endpoints in broncho-alveolar lavage (BAL) were

determined on post-exposure days 7, 28, and 90. The deposition of cobalt (Co) was determined in lungs, lung-associated lymph nodes (LALN), brain, kidneys, testes, and liver.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Exp. duration	Remarks
	LC50	> 241 mg/m ³ air	test mat.		6 h	

Mortality

No mortality

Any other information on results incl. tables

DQ12 exposed rats changes progressed over time and were distinctly different when compared to CNT-induced changes. The changes in BAL of CNT-exposed rats regressed over time. At 11 mg CNT/m³(day 90), most endpoints in BAL were similar to the control groups which matched the clearance pattern of the Cobalt metal impurity used as tracer. Baytubes were cleared from lung tissue over time (measured via the tracer Co).

Histopathology revealed an increased cellularity in the bronchiolo-alveolar region with focal septal thickening and focal septal collagen depositions at 241 mg/m³. Despite a concentration-dependent increase of Co in lung tissue, determinations in the remaining tissues were unobtrusive. Results with CNTs depleted of Co where indistinguishable from non-depleted CNTs.(Pauluhn, 2008 and 2009^{(11a,b)(12)})

Endpoint study record: Hanwha CM-100: Acute toxicity: inhalation.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Secondary source, but well documented and reliable guideline study.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
secondary source	Ministry of Knowledge and Economy (MKE), Korea	2011	Acute inhalation toxicity study of multi-wall carbon nanotubes (MWCNTs) in rat	Ministry of Knowledge and Economy (MKE), 2011. Acute inhalation toxicity study of multi-wall carbon nanotubes (MWCNTs) in rats, tested by KCL(Korea conformity Laboratory) (cited in Multi-walled Carbon Nanotubes (MWCNTs))	KCL(Korea conformity Laboratory)				

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 403 (Acute Inhalation Toxicity)	

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-100

Details on test material

- Name of test material (as cited in study report): Multi-Walled Carbon Nanotubes (MWCNTs)
 - Analytical purity: ca. 95% based on thermogravimetric analysis
 - Other: Identity (purity): Diameter 10~15 nm, length ~200 micron
- Remarks: Manufactured by Hanwha Nanotech Co., Ltd. (Korea)

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Age at study initiation: 8 week old

Administration / exposure

Route of administration

inhalation

Duration of exposure

6 h

Concentrations

Doses: 0,0.15, 0.39, 1.33 mg/m³ 1.33 mg/m³ is, technically, the highest concentration which can be reached by the MWCNT generation Methods employed in this test.

Details on study design

- Duration of observation period following administration: 14 days
- Necropsy of survivors performed: yes

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Exp. duration	Remarks
male/female	LC50	> 1.33 mg/m ³ air			6 h	

Mortality

Mortality was not observed.

Clinical signs

Clinical sign was not observed.

Gross pathology

Necropsy findings, included doses affected, severity and number of animals affected: No pathological finding

Applicant's summary and conclusion**Conclusions**

LC50 of acute inhalation exposure was >1.33 mg/m³.

7.2.3 Acute toxicity: dermal**Endpoint study record: Baytubes: Acute toxicity: dermal.001****Administrative Data**

Purpose flag	supporting study
Study result type	experimental result
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 MaterialScience	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 402 (Acute Dermal Toxicity)	

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Test animals**Species**

rat

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
	LD50	> 2000 mg/kg bw			

Endpoint study record: Graphistrength C100: Acute toxicity: dermal. 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 402)

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**Test type**

standard acute method

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 402 (Acute Dermal Toxicity)	

GLP compliance

yes

Test materials**Test material identity**

Identifier	Identity
	Arkema Graphistrength C100

Details on test material

- Name of test material (as cited in study report): Graphistrength C100
- Other: Micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability

Test animals**Species**

rat

Strain

Sprague-Dawley

Sex

male/female

Administration / exposure**Type of coverage**

semiocclusive

Vehicle

unchanged (no vehicle)

Duration of exposure

24 hrs

Doses

2000 mg/kg

No. of animals per sex per dose

5 males and 5 females

Details on study design

The acute dermal toxicity of Graphistrength C100 (micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability) was evaluated in rats according to OECD 402 and Good Laboratory Practice Regulations. The test item was applied to the skin of one group of ten Sprague-Dawley rats (five males and five females). The application was performed with the test item in its original form at the dose-level of 2000 mg/Kg. The test site was then covered by a semi-occlusive dressing for 24 hours. Clinical signs, mortality and body weight gain were checked for a period of 14 days following the single application of the test item. All animals were subjected to necropsy.

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD0	>= 2000 mg/kg bw	test mat.		

Mortality

No deaths were observed during the study.

Clinical signs

No clinical signs were observed during the study.

A black coloration of the skin was noted in all the animals from day 2 until day 10, in all the males and in one female between day 11 and day 15. This coloration masked the evaluation of cutaneous reactions in all the animals from day 2 until day 6. Crusts were observed in 1/5 males and 2/5 females between day 11 and day 15.

Gross pathology

No apparent abnormalities were observed at necropsy in any animal.

Endpoint study record: Hanwha CM-100: Acute toxicity: dermal.001**Administrative Data**

Purpose flag key study

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. Secondary source, but well documented and reliable guideline deficiencies study.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
secondary source	Ministry of Knowledge and Economy (MKE)	2011	Acute dermal toxicity study of MWCNT in rats	Ministry of Knowledge and Economy (MKE), 2011. Acute dermal toxicity study of MWCNT in rats, tested by KCL(Korea conformity Laboratory) (cited in Multi-walled Carbon Nanotubes (MWCNTs))	KCL(Korea conformity Laboratory)				

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	OECD Guideline 402 (Acute Dermal Toxicity)	

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-100

Details on test material

- Name of test material (as cited in study report): Multi-Walled Carbon Nanotubes (MWCNTs)

- Analytical purity: ca. 95% based on thermogravimetric analysis

- Other:

Identity (purity): Diameter 10~15 nm, length ~200 micron

Remarks: Manufactured by Hanwha Nanotech Co., Ltd. (Korea)

Test animals**Species**

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

Age: 8 weeks old for male and 9 weeks old for female

Administration / exposure**Type of coverage**

semiocclusive

Vehicle

other: DPPC solution(5.5mM D-(+)-glucose + 0.6mg/ml Bovine serum albumin + 0.01 mg/kg DPPC in DPBS)

Details on dermal exposure

The test substance was put on the gauze and the vehicle was added to attach at administration site. Then, it was placed on dermis and tied with non-irritation tapes.

Duration of exposure

24 hrs

Doses

0 and 2,000 mg/kg bw

No. of animals per sex per dose

Five females/dose

Details on study design

- Duration of observation period following administration: 14 days - Necropsy of survivors performed: No data

Results and discussions**Effect levels**

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD50	> 2000 mg/kg bw			

Mortality

Mortality was not observed. Time of death: not applicable

Clinical signs

Clinical sign was not observed.

Applicant's summary and conclusion**Conclusions**

The acute dermal exposure to MWCNT did not induce any sign of toxicity up to 2,000 mg/kg body weight.

7.2.4 Acute toxicity: other routes**Endpoint study record: Graphistrength C100: Acute toxicity: other routes.001****Administrative Data**

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	Tabet L, Bussy C, Setyan A, Simon-Deckers A, Rossi MJ, Boczkowski J, Lanone S.	2011	Coating carbon nanotubes with a polystyrenebased polymer protects against pulmonary toxicity	Particle and Fibre Toxicology, 8, 3-16 (2011)					
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 available		

Test materials

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Details on test material

- Name of test material (as cited in study report): NT-1 (Manufactured by ARKEMA-France. (France))
 - Analytical purity: No data
 - Metallic impurities): Iron: 2.45 %, Aluminium: 3.2%
 - Other
- Specific surface area: 227.4 m²/g, Mean diameter: 12.1 ± 1 nm, Length: 0.1 - 13 µm
 Agglomerate size - mean diameter (%<10 µm): 338 (0.13)

Test animals

Species

mouse

Strain

Balb/c

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Janvier (Le Genest-St-Isle, France)
- Age at study initiation: 7 - 9 weeks
- Weight at study initiation: 22 ± 0.3 g
- Housing: Standard wiretopped cages
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: No data

Administration / exposure

Route of administration

other: Intratracheal instillation

Vehicle

other: Dulbecco's modified Eagle's medium

Details on exposure

The suspension was instilled intratracheally after anaesthesia of the animal with 1.6 mg ketamine (Merial, Lyon, France) plus 300 mg xylazine (Bayer, Puteaux, France).

Doses

10, 100 µg/head

Details on study design

The MWCNTs suspension in sterile Dulbecco's modified Eagle's medium (DMEM) was introduced into mice lungs by intratracheal administration. Male Balb/C mice were treated with either 10 or 100 µg of MWCNTs. Mice were sacrificed at 1, 7, 30, 90 or 180 days post-instillation. Bronchoalveolar Lavage (BAL) and lung were collected. The percentage of macrophages having internalized the different CNT was analyzed. Quantification of the mRNA expression of different genes involved in oxidative stress (SOD-2 and HO-1) and inflammation (TNF- α and MIP-2) was performed by quantitative RT-PCR. In addition, expression of collagen-1 and -3 was analyzed, as markers of interstitial fibrosis. Lung histological analysis was performed in a subset of animals different from that in which BAL analysis and lung gene expression were analyzed.

Statistics

The data were analysed using the non-parametric Kruskal-Wallis test, followed by Dunn's multiple comparison test where appropriate. For all tests, p values smaller than 0.05 were considered significant.

Results and discussions***Mortality***

No data

Clinical signs

No data

Body weight

No data

Gross pathology

No data

Other findings

The results showed significant cellular influx by a single exposure to MWCNTs. Yields of total cells and the number of neutrophils in BAL cells were significantly elevated in MWCNT-treated mice post-treatment day 1. Histological analysis of the lungs at 1 day after instillation demonstrated the presence of micrometric agglomerates of MWCNT at the level of the distal bronchi and the alveolar wall. The agglomerates were preferentially located in the distal bronchi. The late lung response resulted in infiltration and encasement by macrophages to form a connective tissue rich granulomatous inflammation which is typical of the lungs response to an insoluble particle. These lesions were observed from 3 months after instillation and persisted at 6 months post-instillation. No fibrosis was observed. mRNA expression of various genes implicated in oxidative stress, inflammation and fibrosis was quantified in lung homogenates. No modification in the expression of these genes was observed in animals exposed to MWCNT. No modification of GPX-1, SOD-1 or TGF- β s mRNA expression was observed.

Applicant's summary and conclusion**Conclusions**

A single intratracheal instillation with Graphistrength C100 suspension in DME medium at doses of 10 or 100 µg/head to male Balb/C mice also induced pulmonary granulomatous inflammation that persisted at 6 months after instillation

Endpoint study record: Mitsui MWCNT-7: Acute toxicity: other routes. 001**Administrative Data**

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	Wako K, Kotani Y, Hirose A, Doi T, Hamada S.	2010	Effects of preparation methods for multi-walled carbon nanotube (MWCNT) suspensions on MWCNT induced rat pulmonary toxicity	J.Toxicol. Sci., 35 (4), 437-446 (2010)					

Data access

data published

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Test materials**Test material identity**

Identifier	Identity
other:	Mitsui MWNT-7

Test animals**Species**

rat

Strain

other: Crl:CD(SD)

Sex

female

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

- Source: Charles River Laboratories Japan (Japan)
- Age at study initiation: 8 weeks
- Weight at study initiation: 170 - 200g
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature (°C): $22 \pm 2^{\circ}\text{C}$
- Humidity (%): $55 \pm 20\%$
- Photoperiod (hrs dark / hrs light): 12hrs dark/ 12 hrs light

Administration / exposure**Route of administration**

other: intratracheal instillation

Vehicle

other: artificial lung surfactant (ALS)

Details on exposure

The test material suspended in an artificial lung surfactant (ALS) with or without grinding in an agate mortar was administered by single intratracheal instillation to female SD rats at a dose of 5 mg/animal (a dosage volume of 0.5 mL/animal, 12 rats/group).

Doses

5 mg/animal

No. of animals per sex per dose

12 female/dose

Details on study design

The test material suspended in an artificial lung surfactant (ALS) with or without grinding in an agate mortar was administered by single intratracheal instillation to female SD rats at a dose of 5 mg/animal (a dosage volume of 0.5 mL/animal, 12 rats/group). Three rats from each group were subjected to necropsy on days 2, 8, 29 and 92 after instillation. Bronchoalveolar lavage fluid (BALF) was obtained from all rats on each day of necropsy. The difference in the effects on pulmonary toxicity by the amount of agglomerated particles in the instilled suspension was assessed by BALF analysis and lung histopathology.

Results and discussions

Mortality

No data

Clinical signs

No data

Body weight

No data

Gross pathology

No data

Other findings

The MWCNT suspension preparation method with grinding was effective at reducing agglomerates and in increasing uniform dispersion of the fibers. The ground MWCNT induced higher LDH levels and neutrophil ratios in the bronchoalveolar lavage fluid (BALF). There were no remarkable responses in rats in the non-ground MWCNT group, with the exception of inflammatory responses in the early phase. Some histopathological findings varied between rats given the ground MWCNT and non-ground MWCNT. A major difference was an MWCNT-laden macrophage infiltration site in the lung, which were in the alveolus in the ground MWCNT group, and in the interstitium in non-ground MWCNT group. Accordingly, the preparation method with grinding is considered to be effective at reducing agglomerates and ensuring uniform dispersion of the fibers. From these findings, it was concluded that the amount of agglomerates in the suspension is an important factor affecting the pulmonary toxicity of MWCNT.

Any other information on results incl. tables

Table1. Numbers of rats indicating the histopathological lesions in the bronchiolar lymph node and trachea of rats given ground or non-ground multiwall carbon nanotube, Min-U-Sil, or artificial lung surfactant

Lymph node of non-ground multiwall carbon nanotube, Min-U-Sil, or artificial lung surfactant																			
Organ		Control				Ground MWCNT	Non-ground MWCNT		Min-U-Sil										
Findings	Grade	Day2	8	29	92	2	8	29	92		2	8	29	92		2	8	29	92
Lymph node, bronchial																			
Cell infiltration, macrophage	1	0	0	0	0	0	3	0	0		0	0	3	1		0	0	0	0
	2	0	0	0	0	0	0	3	3		0	0	0	1		0	0	0	0
Granuloma	1	0	0	0	0	0	0	0	0		0	0	0	0		0	0	1	0
	2	0	0	0	0	0	0	0	0		0	0	0	0		0	0	2	0
	3	0	0	0	0	0	0	0	0		0	0	0	0		0	0	0	3
Trachea																			
Accumulation, administered substance	1	0	0	0	0	3	0	0	0		2	0	0	0		0	0	0	0
Cell infiltration, inflammatory, mucosa	1	0	0	0	0	1	0	0	0		0	0	0	0		2	0	0	0
Ulcer	1	1	0	0	0	2	0	0	0		1	0	0	0		0	0	0	0
	2	0	0	0	0	3	0	0	0		0	0	0	0		0	0	0	0

	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Hypertrophy, mucosal epithelium	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Cell infiltration, imacrophage, lamina propria, forcal	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0

Table2. Numbers of rats indicating the histopathological lesions in the lung of rats given ground or non-ground multiwall carbon nanotube, Min-U-Sil, or artificial lung surfactant.

Organ		Control					Ground MWCNT					Non-ground MWCNT					Min-U-Sil			
Findings	Grade	Day2	8	29	92		2	8	29	92		2	8	29	92		2	8	29	92
Lung (and brochus)																				
Accumulation, administered substance, alveolus	1	0	0	0	0		0	0	0	0		3	0	0	0		0	0	0	0
	2	0	0	0	0		2	0	0	0		0	0	0	0		0	0	0	0
	3	0	0	0	0		1	0	0	0		0	0	0	0		0	0	0	0
Accumulation, administered substance, brochus	1	0	0	0	0		2	0	0	0		0	0	0	0		0	0	0	0
	2	0	0	0	0		1	0	0	0		2	0	0	0		0	0	0	0
	3	0	0	0	0		0	0	0	0		1	0	0	0		0	0	0	0
Atelectasis, focal	1	0	0	0	0		1	0	0	0		2	0	0	0		0	0	0	0
	2	0	0	0	0		0	0	0	0		1	0	0	0		0	0	0	0
Cell infiltration, inflammatory, focal	1	0	0	0	0		0	0	0	0		0	0	0	0		2	0	2	3
	2	0	0	0	0		3	0	0	0		2	0	0	0		0	0	0	0
	3	0	0	0	0		0	0	0	0		1	0	0	0		0	0	0	0
Cell infiltration, macrophage, alveolus, focal	1	0	0	0	0		3	1	1	0		0	3	1	1		0	1	1	0
	2	0	0	0	0		0	2	2	3		0	0	0	0		0	0	0	3
Cell infiltration, macrophage, interstitium, focal	1	0	0	0	0		0	3	3	3		0	0	1	0		0	0	1	2
	2	0	0	0	0		0	0	0	0		0	3	1	3		0	0	0	0
Erosion, bronchus	1	0	0	0	0		0	0	0	0		2	0	0	0		0	0	0	0
Hyperplasia, lymphoid tissue	1	0	0	0	0		0	0	0	0		1	0	0	0		0	0	0	0
Hypertrophy, alveolar epithelium, focal	1	0	0	0	0		3	0	0	0		0	0	0	0		0	0	0	0
Hypertrophy, bronchial epithelium	1	0	0	0	0		3	1	0	0		2	1	0	0		0	0	0	0

Applicant's summary and conclusion

Conclusions

The MWCNT suspended in an artificial lung surfactant (ALS) with or without grinding in an agate mortar was administered by single intratracheal instillation to rats at a dose of 5 mg/animal. They were subjected to necropsy on days up to 3 months after instillation. The difference in the effects on pulmonary toxicity by the amount of agglomerated particles in the instilled suspension was assessed by BALF analysis and lung histopathology. The ground MWCNT induced higher LDH levels and neutrophil ratios in the BALF. There

were no remarkable responses in rats in the non-ground MWCNT group, with the exception of inflammatory responses in the early phase. Some histopathological findings varied between rats given the ground MWCNT and non-ground MWCNT. A major difference was an MWCNT-laden macrophage infiltration site in the lung, which were in the alveolus in the ground MWCNT group, and in the interstitium in non-ground MWCNT group. Accordingly, the preparation method with grinding is considered to be effective at reducing agglomerates and ensuring uniform dispersion of the fibers. From these findings, it was concluded that the amount of agglomerates in the suspension is an important factor affecting the pulmonary toxicity of MWCNT.

Endpoint study record: Mitsui MWCNT-7: Acute toxicity: other routes. 002

Administrative Data

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	Kobayashi N, Naya M, Ema M, Endoh S, Maru J, Mizuno K, Nakanishi J.	2010	Biological response and morphological assessment of individually dispersed multi-wall carbon nanotubes in the lung after intratracheal instillation in rats	Toxicology, 276, 143-153, (2010)					

Data access

data published

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Test animals

Species

rat

Strain

other: Crl:CD (SD)

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Charles River Laboratories Japan (Japan)
- Age at study initiation: 8 weeks
- Weight at study initiation: 288 - 336 g
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 21 - 24°C
- Humidity (%): 42 - 62%
- Photoperiod (hrs dark / hrs light): 12hrs dark/ 12 hrs light

Administration / exposure

Route of administration

other: intratracheal instillation

Vehicle

other: polyoxyethylene sorbitan monooleate (Tween 80)

Doses

0.04, 0.2, 1 mg/kg bw

Control animals

yes

Details on study design

Biological responses of multi-wall carbon nanotubes (MWCNTs) were assessed after a single intratracheal instillation in rats. The diameter and median length of the MWCNTs used in this study were approximately 60 nm and 1.5 µm, respectively. Groups of male SD rats were intratracheally instilled with 0.04, 0.2, or 1 mg/kg of the individually dispersed MWCNT suspension. After instillation, the bronchoalveolar lavage fluid was assessed for inflammatory cells and markers, and the lung, liver, kidney, spleen, and cerebrum were histopathologically evaluated at 3 day, 1 week, 1 month, 3 month and 6 month post-exposure. Morphology of the instilled MWCNTs in the lungs of rats was assessed using the light microscopy and

transmission electron microscopy (TEM).

Statistics

Statistical significance was determined using multiple comparison tests between the negative control and MWCNT-exposed groups. First, the Bartlett's test was conducted. One-way layout analysis of variance was conducted when the variances were equal. Further, Dunnett's multiple comparison tests were conducted when the differences between the groups were significant. The Kruskal-Wallis test was used when the variances were not equal and Steel's multiple comparison test were conducted when the differences were significant. Statistical significance was determined between the positive and negative control groups using intergroup comparison tests. First, the F-test was conducted: the Student's t-test was used when the variances were equal, and the Aspin-Welch t-test was used when the variances were not equal. Statistical significances were judged at the 0.05 probability level. SAS System version 6.12 was used for all the statistical analyses.

Results and discussions

Mortality

No data

Clinical signs

No data

Body weight

Statistically significant differences in the body weights of experimental animals were not observed any of the MWCNT or crystalline silica-exposed groups and the negative control group at any time point.

Gross pathology

In the groups exposed to 0.2 and 1 mg/kg MWCNTs, brown or black spots were observed in the lung until 1- and 6-month post-exposure, respectively. These spots were considered to be the pigment of the agglomerated MWCNTs.

Other findings

Treatment-related histopathological changes were observed only in the lungs and lung-associated lymph nodes, and not in the other organs (liver, kidney, spleen, and cerebrum). Transient pulmonary inflammatory responses were observed only in the lungs of group of rats exposed to 1 mg/kg MWCNT. Light microscopy examination revealed that MWCNTs deposited in the lungs of the rats were typically phagocytosed by the alveolar macrophages and these macrophages were consequently accumulated in the alveoli until 6 month post-exposure. The 400 TEM images obtained that all MWCNTs were not located in the cells of interstitial tissues. There was no evidence of chronic inflammation, such as angioneurosis or fibrosis, induced by MWCNT instillation. These results suggest that MWCNTs were being processed and cleared by alveolar macrophages.

Applicant's summary and conclusion

Conclusions

Biological responses of multi-wall carbon nanotubes (MWCNTs) were assessed after a single intratracheal instillation in rats. Groups of male SD rats were intratracheally instilled with 0.04, 0.2, or 1 mg/kg of the

individually dispersed MWCNT suspension. After instillation, the bronchoalveolar lavage fluid was assessed for inflammatory cells and markers, and the lung, liver, kidney, spleen, and cerebrum were histopathologically evaluated at 3 day, 1 week, 1 month, 3 month and 6 month post-exposure. Treatment-related histopathological changes were observed only in the lungs and lung-associated lymph nodes, and not in the other organs. Transient pulmonary inflammatory responses were observed only in the lungs of group of rats exposed to 1 mg/kg MWCNT. Light microscopy examination revealed that MWCNTs deposited in the lungs of the rats were typically phagocytosed by the alveolar macrophages and these macrophages were consequently accumulated in the alveoli until 6 month post-exposure. There was no evidence of chronic inflammation, such as angioneogenesis or fibrosis, induced by MWCNT instillation. These results suggest that MWCNTs were being processed and cleared by alveolar macrophages.

Endpoint study record: Mitsui MWNT-7: Acute toxicity: other route. 003

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	Porter, DW., Hubbs, AF., Mercer, RR., Wu., N., Wolfarth, MG., Sriram, K., Leonard, S., Battelli, L., Schwegler-Berry, D., Friend, S., Andrew, M., Chen, BT., Tsuruoka, S., Endo, M. and Castranova, V.	2010	Mouse pulmonary dose-and time course-responses induced by exposure to multi-walled carbon nanotubes	Toxicology, 269, 136-147, (2010)					

Data access

data published

Materials and methods

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 MWNT-7

Test animals**Species**

mouse

Strain

other: C57BL/6J

Sex

male

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

- Source: Jackson Laboratories (Bar Harbor, ME)
- Age at study initiation: 7 weeks old
- Housing: Animals were individually housed in polycarbonate isolator ventilated cage, which were provided HEPA-filtered air. Autoclaved Alpha-Dri vergin cellulose chips and hardwood Beta-chips were used as bedding.
- Diet (e.g. ad libitum): Harlan Teklad Rodent Diet 7913 (ad libitum)
- Water (e.g. ad libitum): Tap water (ad libitum)
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS

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

Administration / exposure**Route of administration**

other: Pharyngeal aspiration

Vehicle

other: Ca²⁺ and Mg²⁺-free phosphate buffered saline, pH7.4, supplemented with 5.5 mM D-glucose, 0.6 mg/mL serum albumin and 0.01 mg/mL 1,2-dipalmitoyl-sn-glycero-3-phosphocholine

Details on exposure

Mice were anesthetized with isoflurane. When fully anestized, the mouse was positioned with its back against a slant board and suspended by the incisor teeth using a rubber band. The mouth was opened, and

the tongue gently pulled aside from the oral cavity. A 50 µl aliquot of sample was pipetted at the base of the tongue, and the tongue was restrained until at least two deep breaths were completed (but for not longer than 15s). Following release of the tongue, the mouse was gently lifted off the board, placed on its left side, and monitored for recovery from anesthesia. Mice received either DM (vehicle control), 10, 20, 40 or 80 µg MWCNT.

Doses

0, 10, 20, 40 or 80 µg

Control animals

yes

Details on study design

- Duration of observation period following administration: The animals were dissected at 1, 7, 28 and 56 days post-exposure.
- Frequency of observations and weighing: No data
- Necropsy of survivors performed: yes
- Other examinations performed: Lung histopathology (to determined doseresponse for histopathologic alterations, the 0, 10, 20, 40, and 80 µg exposure groups were evaluated at 7 and 28 days post-exposure. To evaluate resolution, persistence or progression of the histopathologic alterations, mice from the 0, 20 and 80 µg exposure groups were evaluated at 56 days post-exposure. Four mice were randomly assigned to each group, resulting in a total of 52 mice in this portion of the study.), other: BALF examinations (the 0, 10, 20 and 40 µg exposure groups were evaluated at 1, 7, 28 and 56 days post-exposure. Pulmonary inflammation was assessed by determining BAL polymorphonuclear leukocytes (PMNs). MWCT-induced lung damage was assessed by measuring acellular BAL fluid lactate dehydrogenase activities as a measure of cytotoxicity, and albumin concentrations as a measure of the integrity of the alveolar ? blood barrier.)

Statistics

Statistical comparisons between treatment conditions (e.g., doses) were performed separately for each post-exposure time using analysis of variance (ANOVA) with post hoc t-tests for pair-wise comparison of dose groups. Since variance estimates were different across dose groups, the ANOVA models were estimated using an unequal variance model available from SAS PROC MIXED (Littell et al., 1996). All statistical tests were two tailed with significance level equal to 0.05. Since the pathology data consisted of ordinal scores then comparisons between control and MWCNT doses at each time and comparisons across time for each exposure group were accomplished using two separate one-way nonparametric analyses or variance (ANOVAs). Exact test were used because of the high number of tied values in the data. The nonparametric ANOVA was performed using SAS Proc NPAR1WAY with exact Kruskal ? Wallace tests for multi-group comparisons and exact Wilcoxon tests for post hoc pair-wise comparisons. All statistical tests were two tailed and performed at the 0.05 significance level.

Results and discussions

Other findings

- Histopathology: MWCNT exposure caused rapid development of pulmonary fibrosis by 7 days post-exposure, that granulomatous inflammation persisted throughout the 56-day post-exposure period, and also demonstrated that MWCNT can reach the pleura after pulmonary exposure.
- Other observations: Bronchoalveolar lavage (BAL) studies determined pulmonary inflammation and damage was dose-dependent and peaked at 7 days post-exposure. By 56 days post-exposure, pulmonary

inflammation and damage markers were returning to control levels, except for the 40 µg MWCNT dose, which was still significantly higher than vehicle control.

Applicant's summary and conclusion

Conclusions

In summary, the data reported here indicate that MWCNT exposure rapidly produces significant adverse health outcomes in the lung. Furthermore, the observation that MWCNT reach the pleura after aspiration exposure indicates that more extensive investigations are needed to fully assess if pleural penetration results in any adverse health outcomes.

Executive summary

MWCNT were dispersed in dispersion medium (DM) and male C57BL/6J mice (7 weeks old) received either DM (vehicle control), 10, 20, 40 or 80 µg MWCNT by aspiration exposure. At 1, 7, 28 and 56 days post-exposure, MWCNT-induced pulmonary toxicity was investigated. Bronchoalveolar lavage (BAL) studies determined pulmonary inflammation and damage was dose-dependent and peaked at 7 days post-exposure. By 56 days post-exposure, pulmonary inflammation and damage markers were returning to control levels, except for the 40 µg MWCNT dose, which was still significantly higher than vehicle control. Histopathological studies determined that MWCNT exposure caused rapid development of pulmonary fibrosis by 7 days post-exposure, that granulomatous inflammation persisted throughout the 56-day post-exposure period, and also demonstrated that MWCNT can reach the pleura after pulmonary exposure.

Endpoint study record: Mitsui MWCNT-7: Acute toxicity: other routes. 004

Administrative Data

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	Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Friend S, Castranova V, Porter DW.	2011	Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes	Particle and Fibre Toxicology, 8:21, (2011)					

Data access

data published

Materials and methods

Test animals

Species

mouse

Strain

other: C57BL/6J

Sex

male

Administration / exposure

Route of administration

other: pharyngeal aspiration

Vehicle

other: PBS supplemented with D-glucose, mouse serum albumin and 1,2 dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

Doses

10, 20, 40 or 80 µg/head

Control animals

yes

Details on study design

To address the hypothesis that MWCNTs cause persistent pulmonary pathology, male C57BL/6J mice were exposed by pharyngeal aspiration to 10, 20, 40 or 80 µg of MWCNTs or vehicle (PBS supplemented with D-glucose, mouse serum albumin and 1,2 dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)). Lungs were preserved at 1, 7, 28 and 56 days post-exposure to determine the potential regions and target cells for impact by MWCNT lung burden. Morphometric measurement of Sirius Red staining was used to assess the connective tissue response.

Results and discussions

Mortality

No data

Clinical signs

No data

Body weight

No data

Gross pathology

No data

Other findings

At 56 days post-exposure, 68.7 ± 3.9 , 7.5 ± 1.9 and 22.0 ± 5.1 percent (mean $\pm$ SE, N = 8) of the MWCNT lung burden were in alveolar macrophages, alveolar tissue and granulomatous lesions, respectively. The subpleural tissues contained 1.6% of the MWCNT lung burden. No MWCNTs were found in the airways at 7, 28 or 56 days after aspiration. The connective tissue in the alveolar interstitium demonstrated a progressive increase in thickness over time in the 80 μ g exposure group (0.12 ± 0.01 , 0.12 ± 0.01 , 0.16 ± 0.01 and 0.19 ± 0.01 μ m for 1, 7, 28 and 56 days post-exposure (mean $\pm$ SE, N = 8)). Dose-response determined at 56 days post-exposure for the average thickness of connective tissue in alveolar septa was 0.11 ± 0.01 , $0.14 \pm .02$, 0.14 ± 0.01 , 0.16 ± 0.01 and 0.19 ± 0.01 μ m (mean $\pm$ SE, N = 8) for vehicle, 10, 20, 40 and 80 μ g dose groups, respectively.

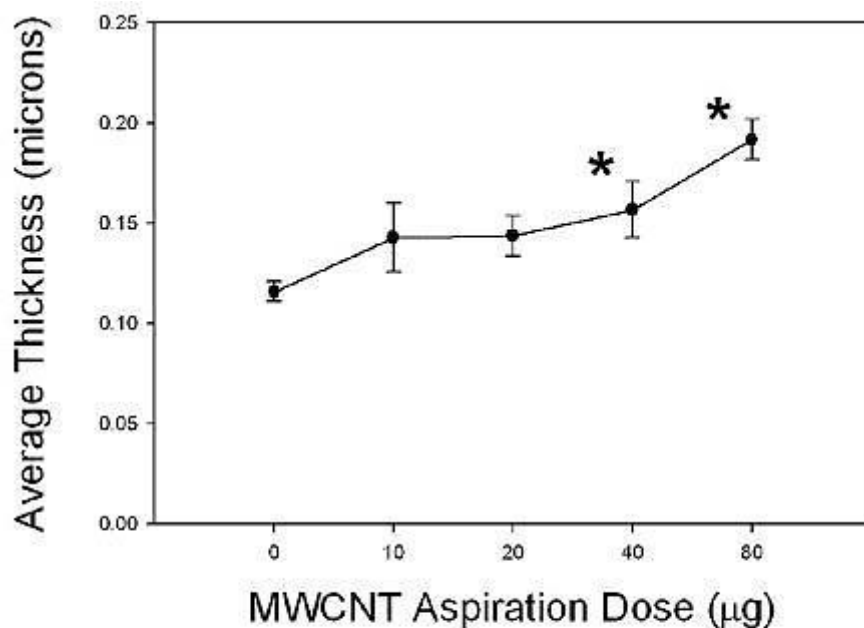
Overall remarks, attachments**Illustration (picture/graph)**

Figure 7 Collagen dose-response at 56 days after exposure to MWCNTs. As shown in Figure 7, there was a progressive increase in the average thickness of the alveolar connective tissue thickness versus aspiration dose of MWCNTs. At a dose of 80 μ g the average thickness was increase by 73% over control. Data are means $\pm$ SE for an N of 8 animals per group. * indicates statistical significance $P < 0.05$ vs. DM -treated control mice.

Applicant's summary and conclusion

Conclusions

The distribution of lung burden was predominately within alveolar macrophages with approximately 8% delivery to the alveolar septa, and a smaller but potentially significant burden to the subpleural tissues. Despite the relatively low fraction of the lung burden being delivered to the alveolar tissue, the average thickness of connective tissue in the alveolar septa was increased over vehicle control by 45% in the 40 µg and 73% in the 80 µg exposure groups. The results demonstrate that MWCNTs have the potential to produce a progressive, fibrotic response in the alveolar tissues of the lungs. However, the increases in connective tissue per µg dose of MWCNTs to the interstitium are significantly less than those previously found for single-walled carbon nanotubes (SWCNTs).

Endpoint study record: Mitsui MWCNT-7: Acute toxicity: other routes. 005

Administrative Data

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	Pacurari M, Qian Y, Porter DW, Wolfarth M, Wan Y, Luo D, Ding M, Castranova V, Guo NL.	2011	Multi-walled carbon nanotube-induced gene expression in the mouse lung: Association with lung pathology						

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Test animals

Species

mouse

Strain

other: C57BL/6 J

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Jackson Laboratories (Bar Harbor, ME)
- Age at study initiation: 7 weeks
- Housing: Individual mice were housed one per cage in polycarbonate isolator ventilated cages, which were provided HEPA-filtered air, with fluorescent lighting from 0700 to 1900 h. Autoclaved Alpha-Dri virgin cellulose chips and hardwood Beta-chips were used as bedding.
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 5 days

ENVIRONMENTAL CONDITIONS

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

Administration / exposure

Route of administration

other: Pharyngeal aspiration

Doses

DM (vehicle control), 10, 20, 40 or 80 µg MWCNT.

No. of animals per sex per dose

eight male/group

Control animals

yes

Details on study design

This study sought to investigate how previously identified lung cancer prognostic biomarkers and the related cancer signaling pathways are affected in the mouse lung following pharyngeal aspiration of well-dispersed MWCNT. MWCNT suspension in dispersion medium was exposed to mice by pharyngeal aspiration. Male C57BL/6 J mice were anesthetized. When fully anesthetized, the mouth was opened, and the tongue gently pulled aside from the oral cavity. A 50µl aliquot of sample was pipetted at the base of the tongue, and the tongue was restrained until at least 2 deep breaths were completed (but for not longer than 15 s). Following release of the tongue, the mouse was gently lifted off the board, placed on its left side, and monitored for recovery from anesthesia. Mice received either DM (vehicle control), 10, 20, 40 or 80 µg MWCNT. At 7 and 56 days post-exposure, a total of 63 identified lung cancer prognostic biomarker genes and major signaling biomarker genes were analyzed in mouse lungs using quantitative PCR assays.

Statistics

To assess the gene expression fold change in each treated group vs. the control group, the gene expression

(Ct value) of three replicates was first averaged for each sample. Second, each gene expression value was normalized using the average expression of 18S for the entire plate, i.e. plate-wise normalization. Third, the fold change between treatment groups vs. the control group was calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Finally, the statistical significance was assessed with two-sample t-tests using software SAS 9.2. In order to assess post-exposure effects over the time series, a two-sample t-test was also performed on the fold change values between two time points for each gene.

Results and discussions

Mortality

No data

Clinical signs

No data

Body weight

No data

Gross pathology

No data

Other findings

At 7 and 56 days post-exposure, a set of 7 genes and a set of 11 genes, respectively, showed differential expression in the lungs of mice exposed to MWCNT vs. the control group. Additionally, these significant genes could separate the control group from the treated group over the time series in a hierarchical gene clustering analysis. Furthermore, 4 genes from these two sets of significant genes, coiled-coil domain containing-99 (Ccdc99), muscle segment homeobox gene-2 (Msx2), nitric oxide synthase-2 (Nos2), and wingless-type inhibitory factor-1 (Wif1), showed significant mRNA expression perturbations at both time points. It was also found that the expression changes of these 4 overlapping genes at 7 days post-exposure were attenuated at 56 days post-exposure. Ingenuity Pathway Analysis (IPA) found that several carcinogenic-related signaling pathways and carcinogenesis itself were associated with both the 7 and 11 gene signatures. Taken together, this study identifies that MWCNT exposure affects a subset of lung cancer biomarkers in mouse lungs.

Overall remarks, attachments

Illustration (picture/graph)

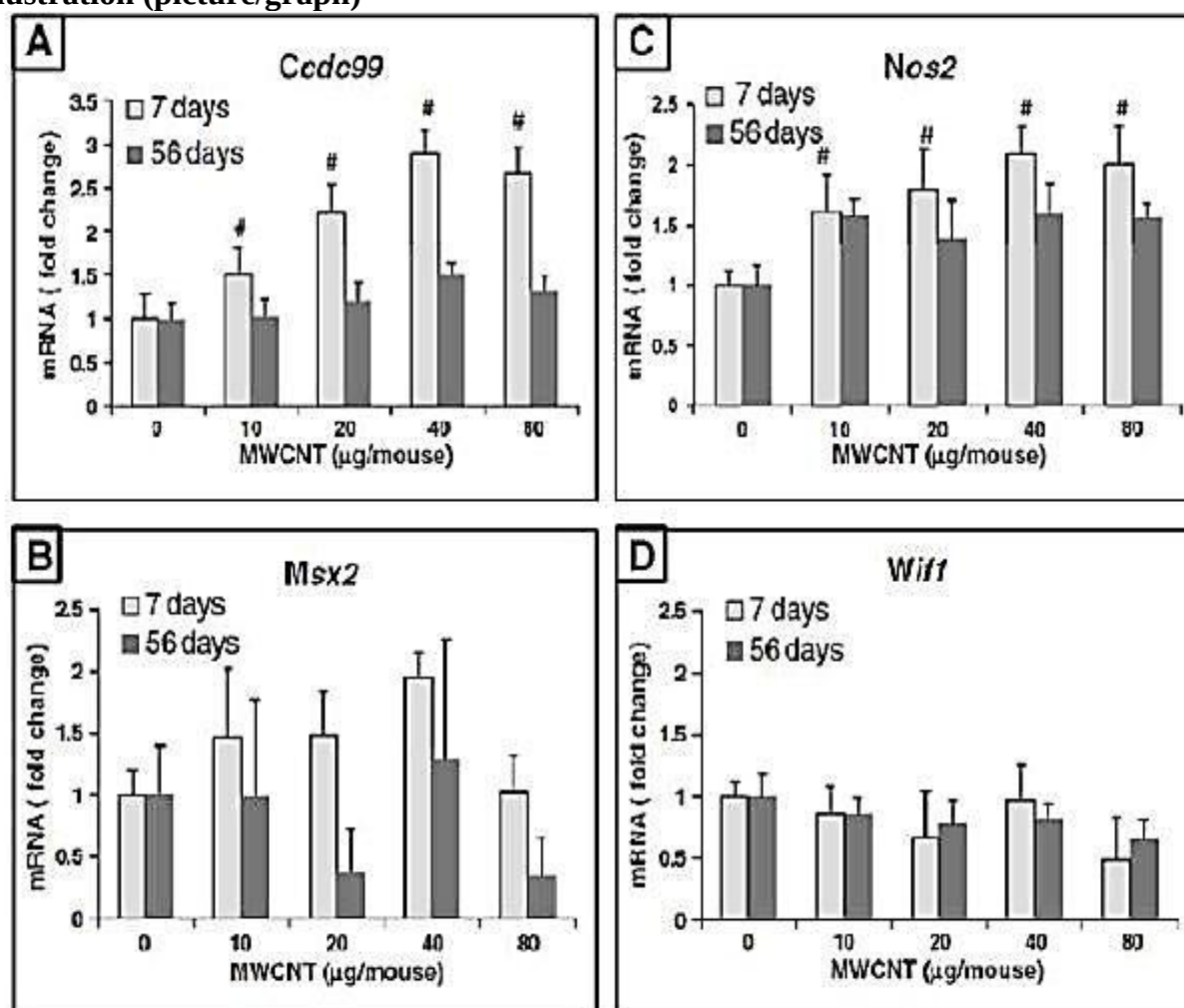


Fig. An overlapping 4-gene biomarker set in the mouse lung between 7- and 11-gene set at 7 and 56 days post-exposure to MWCNT, respectively. A, *Ccdc99*; B, *Msx2*; C, *Nos2*; and D, *Wif1*. Fold-change was calculated after normalization to 18S and relative to control samples. Values given are mean $\pm$ SEM ($p \leq 0.05$, $n=8$). # Significantly different compared to 56 days post-exposure.

Applicant's summary and conclusion

Conclusions

MWCNT suspension in dispersion medium was exposed to mice by pharyngeal aspiration up to 80 µg/mouse. They were sacrificed at 7 and 56 days post exposure, and gene expression profile in the lungs were analyzed using quantitative PCR assays. A set of 7 genes and a set of 11 genes, respectively, showed differential expression in the lungs of mice exposed to MWCNT vs. the control group. Furthermore, 4 genes from these two sets of significant genes, coiled-coil domain containing-99 (*Ccdc99*), muscle segment homeobox gene-2 (*Msx2*), nitric oxide synthase-2 (*Nos2*), and wingless-type inhibitory factor-1 (*Wif1*), showed significant mRNA expression perturbations at both time points. It was also found that the expression changes of these 4 overlapping genes at 7 days post-exposure were attenuated at 56 days post-exposure. Ingenuity Pathway Analysis (IPA) found that several carcinogenic-related signaling pathways

and carcinogenesis itself were associated with both the 7 and 11 gene signatures. Taken together, this study identifies that MWCNT exposure affects a subset of lung cancer biomarkers in mouse lungs.

Endpoint study record: Mitsui MWCNT-7: Acute toxicity: other routes. 006

Administrative Data

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	Erdely A, Liston A, Salmen-Muniz R, Hulderman T, Young SH, Zeidler-Erdely PC, Castranova V, Simeonova PP.	2011	Identification of Systemic Markers from A Pulmonary Carbon Nanotube Exposure	J Occup Environ Med., 53(6 from A Pulmonary Suppl):S80-6, (2011)					

Data access

data published

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Test materials

Test material identity

Identifier	Identity
other:	Mitsui MWNT-7

Test animals

Species

mouse

Strain

other: C57BL/6

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Jackson Laboratory, bar Harbor, ME
- Age at study initiation: 10 weeks
- Housing: ventilated cages in controlled humidity and temperature
- 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

other: pharyngeal aspiration

Doses

40µg/ head

No. of animals per sex per dose

4 - 8 male/group

Control animals

yes

Details on study design

Mice (C57BL/6, 10 weeks old) were exposed by pharyngeal aspiration with 40µg of MWCNT in a total of 50µl, and blood and tissues were harvested at 24 hour, 7 days and 28 days post-exposure. DM (phosphate buffered saline with 0.6 mg/mL serum albumin and 0.01 mg/mL 1,2-dipalmitoylsn-glycero-3-phosphocholine) was used as a vehicle. Whole blood, lung and extrapulmonary tissues (heart, aorta and liver) were subjected to measure gene expression, and blood and bronchoalveolar lavage (BAL) were analyzed for cell differential. Serum protein profiles were also performed.

Results and discussions

Mortality

No data

Clinical signs

No data

Body weight

No data

Gross pathology

No data

Other findings

Exposure to MWCNT results in a measurable systemic inflammatory response. As summarized in Table 1, early effects included increased serum levels of primary cytokines and inflammatory gene expression in blood cells. This was followed by a reduction in the initial inflammatory markers and a predicted acute phase response. Beyond 24 hours post-exposure, a consistent eosinophilic response as well as a series of proteins related to immune activation was evident. At 28 day, serum acute phase proteins with immune function including complement C3, apoprotein A-I and A-II, and alpha2 -microglobulin were increased. In conclusion, MWCNT exposure resulted in measurable systemic markers but lacked specificity to distinguish from other pulmonary exposures.

Any other information on results incl. tables**Table 1 Blood Leukocyte Differentials Examined by Flow Cytometry after MWCNT Exposure**

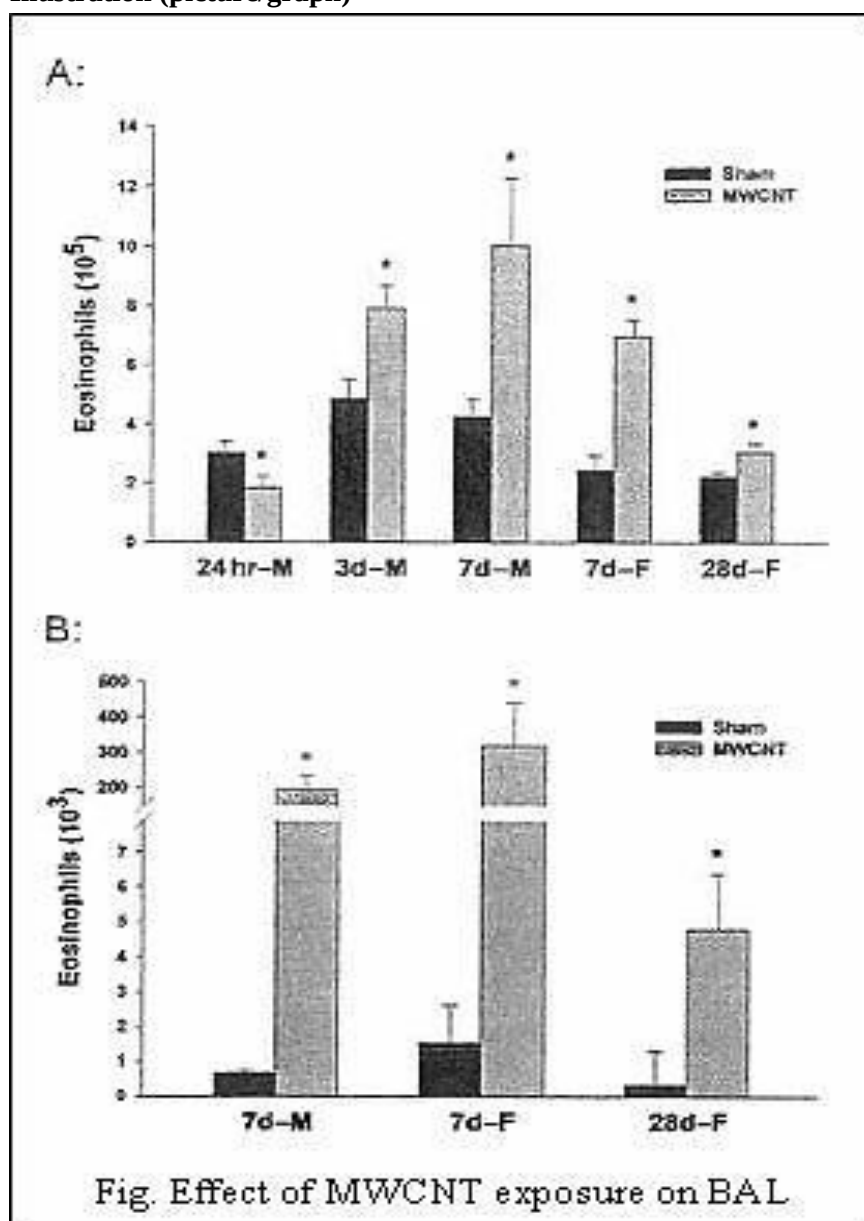
		Total Leukocytes (10 ⁶)	Lymphocytes (10 ⁶)	Monocytes (10 ⁵)	Neutrophils (10 ⁵)
24 hr - Male	Sham (n=8)	11.83±0.88	9.89±0.77 (83%)	5.94 + 0.44 (5%)	10.47 + 0.99 (9%)
	MWCNT (n=8)	6.81±0.35	5.31 + 0.28 (78%)	2.50 + 0.32 (4%)	10.67 + 1.57 (16%)
3 d - Male	Sham (n=4)	16.14±1.45	13.97±1.321 (86%)	8.81 + 1.10 (6%)	7.93 + 0.66 (5%)
	MWCNT (n=4)	13.18±0.63	10.65±0.53 (81%)	7.24 + 0.61 (5%)	10.14 + 0.23 (8%)
7 d - Male	Sham (n=6)	12.25±1.17	9.82 + 1.02 (80%)	9.83 + 1.15 (8%)	10.29 + 1.03 (8%)
	MWCNT (n=7)	14.02±2.14	10.77±1.68 (77%)	10.96 + 1.33 (8%)	11.44 + 1.60 (8%)
7 d - Female	Sham (n=6)	12.45±0.96	11.06±0.92 (89%)	4.09 + 0.41 (3%)	7.32 + 0.59 (6%)
	MWCNT (n=6)	13.88±0.43	11.68±0.33 (84%)	3.69 + 0.29 (3%)	11.33 + 1.47 (8%)
28 d - Female	Sham (n=5)	10.54±1.38	9.17±1.22 (87%)	5.39 + 0.68 (5%)	6.08 + 1.13 (6%)
	MWCNT (n=6)	14.13±2.80	12.18±2.51 (86%)	6.71 + 0.85 (5%)	9.73 + 2.12 (7%)

Table 2 Summary of Systemic markers Following a Single MWCNT Exposure

	< 24 hr	24 hr	> 24 hr
Primary cytokines (eg.6)	-	«()	«
Inflammatory blood gene expression	-	«	«
Coagulatory marker PAI-1	-	-	«
Acute phase proteins (eg. CRP, SAA-1)	«	-	«
BAL eosinophils	«(-)	-	-
Blood eosinophils	«	-	-
Acute phase proteins related to immune activation	nd	nd	-

Overall remarks, attachments

Illustration (picture/graph)



Applicant's summary and conclusion

Conclusions

Mice were exposed by pharyngeal aspiration to 40 ug MWCNT, and harvested 24 hours, 7 days, and 28 days post-exposure for measurements of whole blood, lung and extrapulmonary tissue gene expression, blood and BAL differentials, and serum protein profiling. Early effects included increased inflammatory blood gene expression and serum cytokines followed by an acute phase response. Beyond 24 hours, there was a consistent increase in blood and BAL eosinophils. At 28 day, serum acute phase proteins with immune function were increased. Thus, MWCNT exposure resulted in measurable systemic biomarkers but lacked specificity to distinguish from other pulmonary exposures.

Endpoint study record: Mitsui MWCNT-7: Acute toxicity: other routes. 007**Administrative Data**

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	Osmond-McLeod MJ, Poland CA, Murphy F, Waddington L, Morris H, Hawkins SC, Clark S, Aitken R, McCall MJ, Donaldson K	2011	Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres	Particle and Fibre Toxicology, 8:15, (2011)					

Data access

data published

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Test materials**Test material identity**

Identifier	Identity
other:	Mitsui MWNT-7

Details on test material

- Name of test material (as cited in study report): CNTLONG1 (Mitsui MWCNT-7)

- Other: X607 (Rock wool), LFA (amosite asbestos), LFC (crysotile asbestos) and CNTSW (Sigma SWCNT) were used for comparison.

Test animals**Species**

mouse

Strain

C57BL

Sex

female

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

- Age at study initiation: 8weeks
- Diet (e.g. ad libitum):ad libitum
- Water (e.g. ad libitum): ad libitum

ENVIRONMENTAL CONDITIONS

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

Administration / exposure**Route of administration**

intraperitoneal

Vehicle

other: 0.5% bovine serum albumin

Doses

50 µg/head

No. of animals per sex per dose

Four female/group

Control animals

yes

Details on study design

(in vitro experiment) Gambles solution (simulated biological fluid) was prepared the day before addition to the samples (per litre: 7.12 g NaCl; 1.95 g NaHCO₃; 0.029 g CaCl₂·2H₂O; 0.148 g Na₂HPO₄; 0.079 g Na₂SO₄; 0.212 g MgCl₂·6H₂O; 0.118 g Glycine; 0.152 g Trisodium citrate·2H₂O; 0.18 g Disodium tartrate·2H₂O; 0.172 g Sodium pyruvate; 167 µl lactic acid). Formaldehyde (2 ml/L; 37% in formalin) was added to prevent microbial growth. The pH was adjusted by HCl to 4.5 and then readjusted the next day prior to addition to the samples. For each time-point of interest, four replicates of each test sample (each weighing 1.00-3.00 mg) were weighed (Ohaus AP2500) into 7 ml plastic flat-bottomed Bijou tubes. All samples were then incubated with shaking for up to 24 weeks at 37°C with the Gambles solution refreshed every 3 weeks. The four replicates of each sample were removed from incubation at 0 weeks, 3 weeks, 6 weeks, 10 weeks and 24 weeks and filtered and washed with double-distilled water (ddH₂O) onto pre-weighed PVC filter papers (5.0 µm pore size, 25 mm diameter, SKC Inc). The filtered samples were left to dry protected from dust at room temperature for five days, and then weighed to 0.001 mg (Sartorius MC5-OCE), and percent recoveries relative to recovery at 0 weeks were calculated. (in vivo experiment) Samples that had been incubated in Gambles solution for 0 weeks or 10 weeks were filtered and resuspended in 0.5% bovine serum albumin (BSA): saline at a presumed 100% recovery of mass, and a presumed mass of 50 µg was injected into the peritoneal cavities of female C57Bl/6 mice. Mice were sacrificed at 24 h or 7 d postinjection and the peritoneal cavities were washed and lavage fluid collected.

To identify the presence of an acute inflammatory response, a number of assays were performed: total and differential cell counting to identify possible infiltration of immune cells into the peritoneal release of inflammatory cytokines; and measurement of LDH as a marker for damage to cellular membranes.

Results and discussions

Mortality

No data

Clinical signs

No data

Body weight

No data

Gross pathology

No data

Other findings

(in vitro experiment) After incubation for up to 24 weeks in Gambles solution, our control fibers were recovered at percentages consistent with their known in vitro durabilities and/or in vivo persistence, and three out of the four types of carbon nanotubes tested (single-walled (CNTSW) and multi-walled (CNTTANG2, CNTSPIN)) showed no, or minimal, loss of mass or change in fiber length or morphology when examined by electron microscopy. However, the fourth type [multi-walled (CNTLONG1): Mitsui MWCNT-7] lost 30% of its original mass within the first three weeks of incubation, after which there was no further loss. Electron microscopy of CNTLONG1 samples incubated for 10 weeks confirmed that the proportion of long fibers had decreased compared to samples briefly exposed to the Gambles solution. (in vivo experiment) CNTLONG1 incubated in Gambles solution for 0 weeks induced an acute inflammatory response at 24 h postinjection into mice that did not subside by 7 d, and also induced a strong fibrotic response at 7 d. However, CNTLONG1 that had been incubated in Gambles solution for 10 weeks was less pathogenic in mice, inducing reduced inflammatory and fibrotic responses compared to 0 weeks. This loss of pathogenesis was considered to be accompanied with the loss of mass and fiber shortening in vitro. Conclusions: These results support the view that carbon nanotubes are generally durable but may be subject to bio-modification in a sample-specific manner. They also suggest that pristine carbon nanotubes, either individually or in rope-like aggregates of sufficient length and aspect ratio, can induce asbestos-like responses in mice, but that the effect may be mitigated for certain types that are less durable in biological systems. Results indicate that durable carbon nanotubes that are either short or form tightly bundled aggregates with no isolated long fibers are less inflammogenic in fiber-specific assays.

Overall remarks, attachments

Illustration (picture/graph)

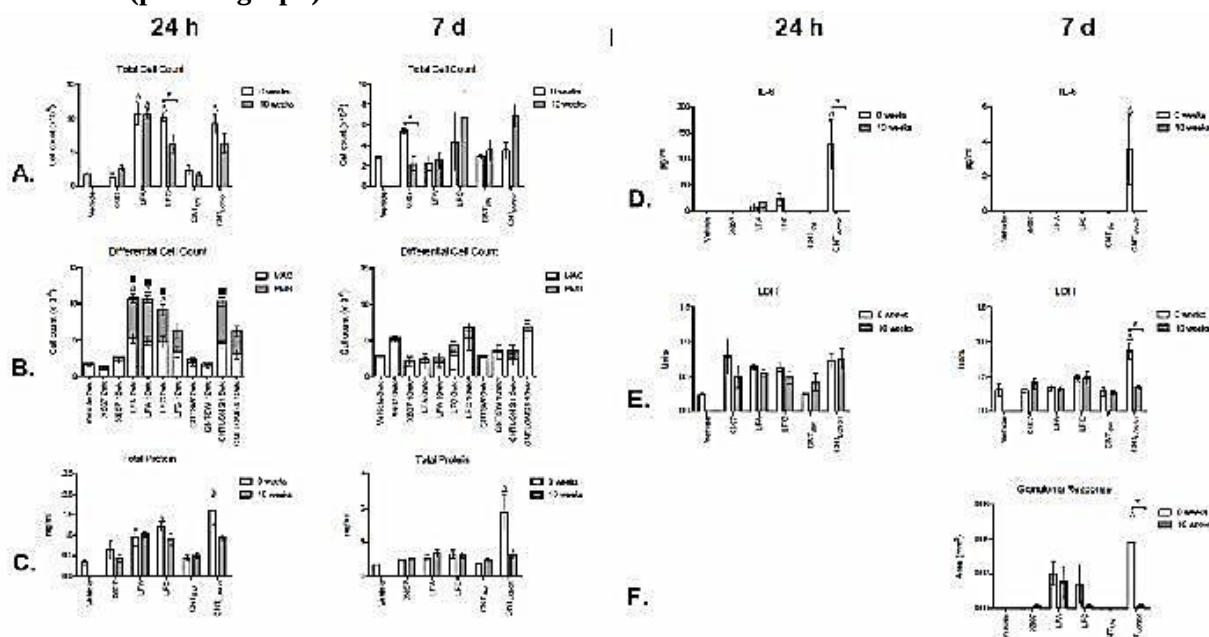


Fig. Inflammatory responses to test samples injected into the peritoneal cavities of mice. Samples of vehicle only, X8007, LFA, LFC, CNTSW and CNT₂₀₁₃, incubated for either 0 weeks or 10 weeks in Gambles solution, were injected into the peritoneal cavities of mice, and inflammatory responses were assessed at 24 h (panels on left) and 7 d (panels on right) post-injection.

Applicant's summary and conclusion

Conclusions

After incubation for up to 24 weeks in Gambles solution, our control fibers were recovered at percentages consistent with their known in vitro durabilities and/or in vivo persistence, and three out of the four types of carbon nanotubes tested (single-walled (CNTSW) and multi-walled (CNTTANG2, CNTSPIN)) showed no, or minimal, loss of mass or change in fiber length or morphology when examined by electron microscopy. However, the fourth type [multi-walled (CNTLONG1)] lost 30% of its original mass within the first three weeks of incubation, after which there was no further loss. Electron microscopy of CNTLONG1 samples incubated for 10 weeks confirmed that the proportion of long fibers had decreased compared to samples briefly exposed to the Gambles solution. This loss of mass and fiber shortening was accompanied by a loss of pathogenicity when injected into the peritoneal cavities of C57Bl/6 mice compared to fibers incubated briefly.

Endpoint study record: Nikkiso MWCNT: Acute toxicity: other routes. 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	Morimoto Y, Hirohashi M, Ogami A, Oyabu T, Myojo T, Todoroki M, Yamamoto M, Hashiba M, Mizuguchi Y, Lee BW, Kuroda E, Shimada M, Wang WN, Yamamoto K, Fujita K, Endoh S, Uchida K, Kobayashi N, Mizuno K, Inada M, Tao H, Nakazato T, Nakanishi J, Tanaka I.	2012	Pulmonary toxicity of well dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation	Nanotoxicology. 6(6):587-599.(2012)					

Materials and methods**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 animals**Species**

rat

Strain

Wistar

Sex

male

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

- Source: Kyudo Co., Ltd (Kumamoto, Japan)

- Age at study initiation: 9 weeks-old
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 1 week

Administration / exposure

Route of administration

other: intratracheal

Vehicle

other: distilled water including 0.05% Triton X

Doses

0, 0.2 mg/rat (0.66 mg/kg), 1.0 mg/rat (3.3 mg/kg)

No. of animals per sex per dose

10 animals per dose

Control animals

yes

Details on study design

- Duration of observation period following administration: The animals were dissected at 3 days, 1 week, 1 month, 3 months, and 6 months after instillation.
- Frequency of observations and weighing: no data
- Necropsy of survivors performed: yes
- Other examinations performed: lung weight, lung histopathology, other: BALF examinations (Cell count, Alkaline phosphatase (ALP) and myeloperoxidase (MPO) released in the BALF supernatant), cytokine-induced neutrophil chemoattractant-1 (CINC-1), CINC-2 and CINC-3 in lung tissue.

Results and discussions

Other findings

Effects on pulmonary inflammation were examined as follows.

- Cell count and ALP release in BALF: In comparison with the negative control group (0.05% Triton X in distilled water), the total cell count was significantly increased in MWCNT 0.2 mg and 1 mg groups at 3 days after intratracheal injection. Significant increases of the neutrophil count were also found at 3 days and 1 month in the MWCNT 1 mg group; a transient increase was found at 3 days in the MWCNT 0.2 mg group. Regarding the amount of released alkaline phosphatase in BALF, significant increases were observed at 1 week and 3 months in the MWCNT 1 mg group, and at 3 days and 3 months in the MWCNT 0.2 mg group.
- CINC concentration in lung and MPO in BALF: The concentration of CINC-1 in lung tissue persistently increased from 3 days to 3 months in the 1 mg MWCNT group compared with that in the negative control group. Meanwhile, the 0.2 mg MWCNT group only revealed a temporary increase at 3 days. Regarding the CINC-2 concentration, there were significant increases at 1 month and 3 months in 1 mg MWCNT groups. In the 0.2 mg MWCNT group, a significant increase was not found throughout the observation period. As

for CINC-3 concentration, there were not significant increases in either the 0.2 or 1 mg MWCNT group. The MPO concentration showed significant increases over the negative control from 3 days to 1 month in the 1 mg MWCNT group; significant increases in MPO were found at 3 days, 1 month and 3 months in the 0.2 mg group.

- Histopathology in lung: In the group to which 1 mg of MWCNT was administered, neutrophils, eosinophils and alveolar macrophages were constantly infiltrating from alveolar ducts and alveolar spaces to terminal bronchioles. This continued for at least 6 months, although the extent of infiltration decreased as time elapsed during the observation period. However, at the 3 and 6 months, neutrophils and eosinophils were almost not observed; infiltration was mainly by alveolar macrophages. Small granulomatous lesion and transient collagen deposition were also observed. Regarding the group to which 0.2 mg of MWCNT was administered, granulomatous lesions were not observed. Only slight and transient infiltration of inflammatory cells was observed.

Applicant's summary and conclusion

Conclusions

Multi-walled carbon nanotubes (MWCNTs), dispersed in suspensions consisting mainly of individual tubes, were used for intratracheal instillation study. Rats intratracheally received a dose of 0.2 mg, or 1 mg of MWCNTs and were sacrificed from 3 days to 6 months. MWCNTs induced a pulmonary inflammation, as evidenced by a transient neutrophil response in the low-dose groups, and presence of small granulomatous lesion and persistent neutrophil infiltration in the high-dose groups. The results of this study show that well-dispersed MWCNT can produce pulmonary lesions, including inflammation.

7.3 Irritation / corrosion

7.3.1 Skin irritation / corrosion

Endpoint study record: Baytubes: Skin irritation / corrosion.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
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 MaterialScience	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 404 (Acute Dermal Irritation / Corrosion)	

Test materials

Test material identity

Identifier	Identity
other:	Baytubes

Applicant's summary and conclusion

Conclusions

non-irritant

Endpoint study record: Baytubes: Skin irritation / corrosion.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
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 MaterialScience	METI Japan and U.S. EPA	2011	draft dossier development plan for MWCNT						

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD TG 431 (in vitro dermal corrosion)	

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Applicant's summary and conclusion**Conclusions**

Not corrosive

Endpoint study record: Graphistrength C100: Skin irritation / corrosion.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 404)

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 method**

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	

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: Arkema Graphistrength	

Details on test material

- Name of test material (as cited in study report): Graphistrength C100
- Other: Micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability

Test animals**Species**

rabbit

Strain

New Zealand White

Test system**Type of coverage**

semiocclusive

Preparation of test site

shaved

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL

- Amount(s) applied (volume or weight with unit): 0.5 g

Duration of treatment / exposure

4 hrs

Observation period

1, 24, 48, 72 hrs, 4, 5, 6, 7, 8 days after exposure.

Number of animals

3 rabbits

Control animals

no

Details on study design**TEST SITE**

- Area of exposure: one flank

REMOVAL OF TEST SUBSTANCE

- Time after start of exposure: 4hrs

SCORING SYSTEM:**Any other information on materials and methods incl. tables**

The potential of Graphistrength C100 (micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability) to induce skin irritation was evaluated in rabbits according to OECD No. 404 and Good Laboratory Practice Regulations. The test item was first applied for periods of 3 minutes, 1 hour and 4 hours to a single male New Zealand White rabbit. Since the test item was neither severely irritant nor corrosive on this first animal, it was then applied simultaneously for 4 hours to two other animals. A single dose of 500 mg of the test item in its original form was applied to the closely-clipped skin of one flank. The test item was held in contact with the skin by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately 1 hour, 24, 48 and 72 hours after removal of the dressing and then daily until reversibility of cutaneous reactions (day 8). The mean values of the scores for edema were calculated for each animal.

Results and discussions***Irritant/corrosive response data***

After a 3-minute exposure (one animal), no cutaneous reactions were observed. A grey coloration of the skin was noted from day 1 until day 4. After a 1-hour exposure (one animal), a grey coloration of the skin was noted from day 1 until day 4. This coloration could have masked a possible very slight erythema on day 1. No cutaneous reactions were observed from day 2 until day 4. After a 4-hour exposure (three animals), a grey coloration of the skin was noted in all the animals all over the observation period. This coloration could have masked a possible very slight or well-defined erythema from day 1 until day 2, 3 or 4. A very slight erythema was noted in 1/3 animals on days 4 and 5. A dryness of the skin was noted in 2/3 animals between day 4 and day 7. Due to the coloration of the skin, the mean scores over 24, 48 and 72 hours for each animal were not calculable for erythema. For edema, the mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0 and 0.0. However, taking into account the possible erythema masked by the coloration, the maximal mean values over 24, 48 and 72 hours for erythema could be: 0.3, 1.0 and 1.3.

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

slightly irritating

Conclusions

Graphistrength C100 (micronized), induced a skin coloration preventing conclusions about its skin irritation potential. However, taking into account the possible maximal mean values for erythema, the test item Graphistrength C100 micronized could be considered as slightly irritating.

Endpoint study record: Mitsui MWNT-7: Skin irritation / corrosion.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 TG404

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema, M., Matsuda, A., Kobayashi, N., Naya, M. and Nakanishi, J.	2011	Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotubes	Regulatory Toxicology and Pharmacology, 61, 276-281, (2011)	Ina Research				

Data access

data published

Materials and methods**Type of method**

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	

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): 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: 60 nm

Test animals

Species

rabbit

Strain

other: Kbl:NZW

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Kitayama Labes Co. Ltd, Japan
- Age at study initiation: 17 weeks old
- Weight at study initiation: 2.7 - 3.6 kg bw
- Housing: Animals were housed individually in standard aluminum cages.
- Diet (e.g. ad libitum): LRC4; Oriental Yeast Co. Ltd., Japan (ad libitum)
- Water (e.g. ad libitum): Tap water (ad libitum)
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 19.0 - 25.0 °C
- Humidity (%): 40 - 70%
- Air changes (per hr): 15 - 21 times/hr
- Photoperiod (hrs dark / hrs light): 12 hrs dark/ 12 hrs light

Test system

Type of coverage

occlusive

Preparation of test site

shaved

Vehicle

other: Olive oil

Amount/concentration applied

TEST MATERIAL

- Amount(s) applied: 0.5g
- Concentration : 2%

Duration of treatment / exposure

4 h

Observation period

1, 24, 48 and 72 hrs

Number of animals

3 rabbits

Control animals

not required

Any other information on materials and methods incl. tables

The test was done using three male Kbl:NZW rabbits of 17 weeks of age, following OECD TG 404. 0.5 g of 2 wt% of CNTs solution, which is prepared at the maximum concentration allowing a uniform mixture in a media of non-irritating olive oil, was applied on a lint of 2.5 x 2.5 cm. The lint was occlusive dressed to the pelage removed back skin of the rabbits. In four hours the lint was removed and the adhered part was cleaned with warm and wet gauze. In 1, 24, 48 and 72 hours after the lint removal, the red macular, the crust formation, the edematous formation, etc. are observed. The influence is evaluated by a primary stimulation index rating slight as 0-2, moderate as 2-5, and severe as over 5.

Results and discussions**Irritation / corrosion results**

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
primary dermal irritation index (PDII)			ca. 0			

Irritant/corrosive response data

No indicative of abnormality was observed for all the rabbits.

Overall remarks, attachments**Attached full study report**

Attached full study report
③ M-SWCNT irri-sensitize (Reg.Tox.Pharm.P276-281).pdf / 565.61 KB (application/pdf): Appendix 1

Illustration (picture/graph)

Materials	N-SWCNTs	SG-SWCNTs	N-MWCNTs	MWNT-7
<i>Acute dermal irritation study</i>				
Dermal responses (mean score) ^a				
1 h after removal of patches	0	0	0	0
24 h after removal of patches	0	0	1.0	0
48 h after removal of patches	0	0	0.7	0
72 h after removal of patches	0	0	0	0
Primary irritation index (PII) ^b	0	0	0.6	0

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

not irritating

Criteria used for interpretation of results

not specified

Endpoint study record: Nanocyl NC7000: Skin irritation / corrosion.001**Administrative Data**

Purpose flag	supporting study
Study result type	experimental result
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 method**

in vitro

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD TG 431 (in vitro dermal corrosion)	

Test materials**Test material identity**

Identifier	Identity
other:	Nanocyl NC 7000

Applicant's summary and conclusion**Conclusions**

No irritation and no corrosion

Endpoint study record: Nikkiso: Skin irritation / corrosion.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 TG404

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema, M., Matsuda, A., Kobayashi, N., Naya, M. and Nakanishi, J.	2011	Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotubes	Regulatory Toxicology and Pharmacology 61, 276-281, (2011)					
study report	AIST	2010			Ina Research	ZT10128			2010-09-23

Materials and methods**Type of method**

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	

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 MWCNT

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

Test animals**Species**

rabbit

Strain

other: Kbl:NZW

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

- Source: Kitayama Labes Co. Ltd, Japan
- Age at study initiation: 17 weeks old
- Weight at study initiation: 2.7 - 3.6 kg bw
- Housing: Animals were housed individually in standard aluminum cages.
- Diet (e.g. ad libitum): LRC4; Oriental Yeast Co. Ltd., Japan (ad libitum)
- Water (e.g. ad libitum): Tap water (ad libitum)
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 19.0 - 25.0 °C
- Humidity (%): 40 - 70%
- Air changes (per hr): 15 - 21 times/hr
- Photoperiod (hrs dark / hrs light): 12 hrs dark/ 12 hrs light

Test system**Type of coverage**

occlusive

Preparation of test site

shaved

Vehicle

other: olive oil

Amount/concentration applied

TEST MATERIAL

- Amount(s) applied: 0.5g
- Concentration : 1%

Duration of treatment / exposure

4 h

Observation period

1, 24, 48 and 72 hrs

Number of animals

3 rabbits

Control animals

not required

Details on study design

The test was done using three male Kbl: NZW rabbits of 17 weeks, following OECD TG 404. 0.5g of 1 wt% of CNTs solution, which is prepared at the maximum concentration allowing a uniform mixture in a media of non-irritating olive oil, was applied on a lint of 2.5 x 2.5 cm. The lint was occlusive dressed to the pelage removed back skin of the rabbits. In four hours the lint was removed and the adhered part was cleaned with warm and wet gauze. In 1, 24, 48 and 72 hours after the lint removal, the erythema, the eschar formation, the edema formation, etc. are observed. The influence is evaluated by a primary stimulation index rating slight as 0-2, moderate as 2-5, and severe as over 5.

Results and discussions**Irritation / corrosion results**

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
primary dermal irritation index (PDII)	mean		ca. 0.6			

Irritant/corrosive response data

No indicative of abnormality was observed for all the rabbits. However, three rabbits showed mild erythema (primary stimulation index was rated 0.6) after 24 hours from the lint removal. The erythema disappeared within 48 hours after removal for one case and within 72 hours for two cases.

Any other information on results incl. tables**Table 1 Primary skin irritation study of Carbon nanotube in rabbits Observation of skin**

Animal No.	Skin signs	Score of skin reaction ^{a)}						
		First test			After removal of patches			
		3 min	1 h	4 h	60 min	24 h	48 h	72 h
DT1M01	Erythema and eschar formation	0	0	0	0	1	1	0
	Oedema formation	0	0	0	0	0	0	0
	Total	0	0	0	0	1	1	0
DT1M02	Erythema and eschar formation	-	-	-	0	1	1	0
	Oedema formation	-	-	-	0	0	0	0
	Total				0	1	1	0
DT1M03	Erythema and eschar formation	-	-	-	0	1	0	0
	Oedema formation	-	-	-	0	0	0	0
	Total				0	1	0	0
Mean					0.0	1.0	0.7	0.0
P.I.I. ^{b)}						0.6		

-: Not examined

a) Evaluations of skin reactions were scored as follows [OECD TG 404 (2002)]

Erythema and Eschar Formation;

0 = No erythema, 1 = Very slight erythema (barely perceptible), 2 = Well defined erythema,

3 = Moderate to severe erythema, 4 = Severe erythema (beef redness) to eschar formation preventing grading of erythema

Oedema Formation;

0 = No oedema, 1 = Very slight oedema (barely perceptible), 2 = Slight oedema (edges of area well defined by definite raising), 3 = Moderate oedema (raised approximately 1 mm), 4 = Severe oedema (raised more than 1 mm and extending beyond area of exposure)

b) P.I.I. (Primary Irritation Index) = (24 h mean scores + 48 h mean scores + 72 h mean scores) / 3

Criteria for primary skin irritation:

0 < P.I.I. ≤ 2 = Mildly irritating, 2 < P.I.I. ≤ 5 = Moderately irritating, 5 < P.I.I. = Severely irritating

When no skin reactions were observed in any application site during the observation period, the test formulation was judged to be "Not irritating".

Overall remarks, attachments**Attached full study report**

Attached full study report
③ M-SWCNT irri-sensitize (Reg.Tox.Pharm.P276-281).pdf / 565.61 KB (application/pdf): Appendix 1

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

slightly irritating

Criteria used for interpretation of results

not specified

7.3.2 Eye irritation

Endpoint study record: Baytubes: Eye irritation.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
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 MaterialScience	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 405 (Acute Eye Irritation / Corrosion)	

Test materials

Test material identity

Identifier	Identity
other:	Baytubes

Applicant's summary and conclusion

Conclusions

slight irritant

Endpoint study record: Graphistrength C100: Eye irritation.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Adequate coherence between data and conclusions. GLP and guideline compliant.

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 method**

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 405 (Acute Eye Irritation / Corrosion)	

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:	Arkema Graphistrength C100

Details on test material

- Name of test material (as cited in study report): Graphistrength C100
- Other: Mean agglomerate size 473 µm

Test animals**Species**

rabbit

Strain

New Zealand White

Test system**Number of animals**

three rabbits

Details on study design**REMOVAL OF TEST SUBSTANCE**

- Washing (if done): washed with a sterile isotonic saline solution (0.9% NaCl)

Any other information on materials and methods incl. tables

The potential of Graphistrength C100 (mean agglomerate size 473 μm), to induce irritation following a single ocular administration to rabbits was evaluated according to OECD (No. 405, 24th April 2002) and Commission Regulation (EC) (No. 440/2008, B.5, 30 May 2008) guidelines. The study was conducted in compliance with the principles of Good Laboratory Practice. Graphistrength C100, was first administered to a single male New Zealand White rabbit. Since the test item was irritant on the first animal, it was then evaluated in sequential manner on two other animals. The quantity of the test item administered was 0.1 g. The test item was introduced into the conjunctival sac of the left eye. The right eye, which remained untreated, served as control. Approximately 24 hours after instillation of the test item, both eyes were rinsed with a sterile isotonic saline solution (0.9% NaCl). Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration and then daily until the reversibility of the ocular reactions (two animals) or until the end of the observation period (one animal). The mean values of the scores for chemosis, redness of the conjunctiva, iris lesions and corneal opacity were calculated for each animal. Body weight was recorded at the beginning and the end of the observation period. On completion of the observation period, the animals were sacrificed then discarded without macroscopic post-mortem examination.

Results and discussions**Overall irritation / corrosion results**

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
chemosis score	mean	24hr	3			
chemosis score	mean	48hr	2			
chemosis score	mean	72hr	2.7			
conjunctivae score	mean	24hr	2.3			
conjunctivae score	mean	48hr	2			
conjunctivae score	mean	72hr	2.7			
iris score	mean	24hr	0.3			
iris score	mean	48hr	0.7			
iris score	mean	72hr	1			
cornea score	mean	24hr	3			
cornea score	mean	42hr	1.7			
cornea score	mean	72hr	2.3			

Irritant/corrosive response data

First animal: a marked or severe chemosis was noted from day 1 until day 3, followed by a slight or moderate from day 4 until the end of the observation period (except on day 12 where a marked chemosis

was observed). A severe redness of the conjunctiva was noted on days 1 and 2, then a slight or moderate redness of conjunctiva was observed from day 3 until day 21 (except on days 12 and 13, where a severe redness of the conjunctiva was noted). A clear discharge was noted on days 1, 3 and 4 and then from day 11 until day 14. Scoring of iris lesions and corneal opacity was masked on day 1 by residual test item. Iris lesions (grade 1) were noted on days 2 and 11. A marked opacity of cornea, which covered the whole area of the cornea on day 2, was observed from day 2 until day 4. Then, a slight or moderate opacity of the cornea was noted from day 5 until day 21. Neovascularisation was noted from day 4 until day 7 and from day 11 until day 15. Second animal: a marked chemosis was noted on days 1 and 2, followed by a slight or moderate chemosis from day 3 until day 7. On day 1, a moderate redness of the conjunctiva was observed, followed by a severe redness of the conjunctiva on day 2. Then, a slight or moderate redness of the conjunctiva was noted from day 3 until day 7. A clear discharge was observed on days 1 and 3. Iris lesions were noted on days 2 and 3. A slight or moderate corneal opacity was noted from day 2 until day 4. Third animal: a marked or severe chemosis was noted on days 1 and 2. Then, a slight or moderate chemosis was observed from day 3 until day 10. A severe redness of the conjunctiva was noted from day 1 until day 3, followed by a slight or moderate redness of the conjunctiva from day 4 until day 9. A clear discharge was recorded on days 1 and 3. Scoring of iris lesions and corneal opacity was masked on day 1 by residual test item. Iris lesions were noted from day 2 until day 4. A marked opacity of cornea, which covered the whole area of the cornea, was observed on day 2. Then, a slight or moderate opacity of the cornea was noted from day 3 until day 7. The mean scores calculated for each animal over 24, 48 and 72 hours were 3.0, 2.0 and 2.7 for chemosis, 2.3, 2.0 and 2.7 for redness of the conjunctiva, 0.3, 0.7 and 1.0 for iris lesions and 3.0, 1.7 and 2.3 for corneal opacity. GraphistrengthC100 (mean agglomerate size 473µm) was irritating when administered by ocular route to rabbits

Applicant's summary and conclusion

Conclusions

Eye irritation was observed in an in vivo assays with Graphistrength C100. Corneal opacity was observed in all animals. However, no corneal opacification was observed in a Bovine corneal opacity and permeability (BCOP) Assay with Graphistrength C100, indicating that the corneal opacification, and probably the other lesions (conjunctive and iris), are not from a cytotoxic origin but secondary to an abrasive (mechanical) action.

Endpoint study record: Graphistrength C100: Eye irritation.002

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Adequate coherence between data and conclusions. GLP and guideline compliant.

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 method**

in vitro

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD TG 437	

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:	Arkema Graphistrength C100

Details on test material

- Name of test material (as cited in study report): Graphistrength C100
- Other: Mean agglomerate size 473 µm

Test animals**Species**

other: Corneas obtained from freshly slaughtered calves

Test system

Details on study design

The potential of Graphistrength C100 (mean agglomerate size 473 µm) to cause corrosion/severe irritation by using the Bovine corneal opacity and permeability (BCOP) Assay was examined in agreement with OECD guideline no. 437 (adopted 7 September 2009) and Good Laboratory Practice Regulations. Corneas obtained from freshly slaughtered calves were mounted in corneal holders. Both chambers of the corneal holder were filled with complemented MEM culture media (cMEM) and preincubated for 1 hour at 32°C. Three corneas were used for each treated series (test item, positive and negative controls). Before the treatment, a first opacity measurement was performed using an opacitometer. For the treatment, the test item was used in its original form (neat). The test item was tested in a single experiment using a treatment time of 4 hours. At the completion of the treatment period, the test item was removed from the front opening of the anterior chamber and the epithelium was rinsed. After the second opacity measurement, the medium of the anterior chamber was removed and filled with a fluoresceine solution. The holders were then incubated vertically for 90 minutes at 32°C. At the end of the incubation, the optical density of the solution from the posterior chamber of each holder was measured in order to determine the permeability of the cornea. Then the cornea was observed for opaque spots and other irregularities.

Results and discussions

Irritant/corrosive response data

No notable opaque spots or irregularities were observed on negative control corneas following the treatment. Residual test item was observed on corneas treated with the test item. The test item In Vitro Irritancy Score (IVIS) was: 0.2. As the test item Graphistrength C100 (mean agglomerate size 473 µm) induced an IVIS < 55.1.

Applicant's summary and conclusion

Conclusions

It was not identified as ocular corrosive or severe irritant.

Endpoint study record: Mitsui MWNT-7: Eye irritation.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 TG405
Data source	

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema, M., Matsuda, A., Kobayashi, N., Naya, M. and Nakanishi, J.	2011	Evaluation of Dermal and eye irritation and skin sensitization due to carbon nanotubes	Regulatory Toxicology and Pharmacology, 61, 276-281, (2011)	Ina research				

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 405 (Acute Eye Irritation / Corrosion)	

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): 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: 60 nm

Test animals

Species

rabbit

Strain

other: Kbl:NZW

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Kitayama Labes Co. Ltd, Japan
- Age at study initiation: 11 weeks old
- Weight at study initiation: 2.2 - 2.5 kg bw
- Housing: Animals were housed individually in standard aluminum cages.
- Diet (e.g. ad libitum): LRC4; Oriental Yeast Co. Ltd., (ad libitum)
- Water (e.g. ad libitum): Tap water (ad libitum)
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 19.0 - 25.0 °C
- Humidity (%): 40 - 70%
- Air changes (per hr): 15 21 times/hr
- Photoperiod (hrs dark / hrs light): 12 hrs dark/ 12 hrs light

Test system**Vehicle**

other: Olive oil

Amount/concentration applied**TEST MATERIAL**

- Amount(s) applied : 0.1 mL
- Concentration : 1%

Duration of treatment / exposure

One time

Observation period

1, 24, 48 and 72 hrs

Number of animals

3 rabbits

Control animals

not required

Any other information on materials and methods incl. tables

The test was done using three male Kbl:NZW rabbits of 11 weeks of age, following OECD TG 405. 0.1ml of 1.0 wt% CNTs solution, which was prepared at the maximum concentration allowing the instillation into the eyes in a media of non-irritating olive oil, was dropped into the conjunctival sac of left eye. The test specie was rinsed off with warm saline from cornea and conjunctival sac in an hour. In 1, 24, 48 and 72 hours after the instillation, the irritation to the eye was checked.

Results and discussions**Overall irritation / corrosion results**

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
cornea score	mean	1, 24, 48, 72 hrs	ca. 0			
iris score	mean	1, 24, 48, 72 hrs	ca. 0			
chemosis score	mean	1, 24, 48, 72 hrs	ca. 0			
conjunctivae score	mean	1, 24, 48, 72 hrs	ca. 0			

Overall remarks, attachments**Attached full study report**

Attached full study report
③ M-SWCNT irri-sensitize (Reg.Tox.Pharm.P276-281).pdf / 565.61 KB (application/pdf): Appendix 1

Illustration (picture/graph)

Materials	N-SWCNTs	SG-SWCNTs	N-MWCNTs	MWNT-7
<i>Acute eye irritation study</i>				
Corneal opacity (mean score) ^f				
1 h after ocular instillation	0	0	0	0
24 h after ocular instillation	0	0	0	0
48 h after ocular instillation	0	0	0	0
72 h after ocular instillation	0	0	0	0
Conjunctival redness (mean score) ^g				
1 h after ocular instillation	0	0	1,0	0
24 h after ocular instillation	0	0	0	0
48 h after ocular instillation	0	0	0	0
72 h after ocular instillation	0	0	0	0
Abnormality of the iris (mean score) ^g				
1 h after ocular instillation	0	0	0	0
24 h after ocular instillation	0	0	0	0
48 h after ocular instillation	0	0	0	0
72 h after ocular instillation	0	0	0	0
Chemosis (mean score) ^g				
1 h after ocular instillation	0	0	0	0
24 h after ocular instillation	0	0	0	0
48 h after ocular instillation	0	0	0	0
72 h after ocular instillation	0	0	0	0
Corneal epithelium damage (mean score) ^h	0	0	0	0

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

not irritating

Criteria used for interpretation of results

not specified

Conclusions

No indicative of eye irritation was observed.

Endpoint study record: Nikkiso: Eye irritation.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 TG405

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema, M., Matsuda, A., Kobayashi, N., Naya, M. and Nakanishi, J.	2011	Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotubes	Regulatory Toxicology and Pharmacology, 61, 276-281, (2011)					
study report	AIST	2010			Ina Research	ZT10129			2010-09-23

Materials and methods**Type of method**

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 405 (Acute Eye Irritation / Corrosion)	no

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 MWCNT

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

Test animals

Species

rabbit

Strain

other: Kbl:NZW

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Kitayama Labes Co. Ltd, Japan
- Age at study initiation: 11 weeks old
- Weight at study initiation: 2.2 - 2.5 kg bw
- Housing: Animals were housed individually in standard aluminum cages.
- Diet (e.g. ad libitum): LRC4; Oriental Yeast Co. Ltd., (ad libitum)
- Water (e.g. ad libitum): Tap water (ad libitum)
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 19.0 - 25.0 °C
- Humidity (%): 40 - 70%
- Air changes (per hr): 15 - 21 times/hr
- Photoperiod (hrs dark / hrs light): 12 hrs dark/ 12 hrs light

Test system

Vehicle

other: Olive oil

Amount/concentration applied

TEST MATERIAL

- Amount(s) applied : 0.1 mL
- Concentration : 0.25%

Duration of treatment / exposure

One time

Observation period

1, 24, 48 and 72 hrs

Number of animals

3 rabbits

Control animals

not required

Any other information on materials and methods incl. tables

The test was done using three male Kbl:NZW rabbits of 11 weeks of age, following OECD TG 405. 0.1ml of 0.25 wt% CNTs solution, which was prepared at the maximum concentration allowing the instillation into the eyes in a media of non-irritating olive oil, was dropped into the conjunctival sac of left eye. The test specie was rinsed off with warm saline from cornea and conjunctival sac in an hour. In 1, 24, 48 and 72 hours after the instillation, the irritation to the eye was checked.

Results and discussions**Overall irritation / corrosion results**

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
cornea score	mean	1, 24, 48, 72hrs	ca. 0	0		
iris score	mean	1, 24, 48, 72hrs	ca. 0	0		
chemosis score	mean	1, 24, 48, 72hrs	ca. 0	0		
conjunctivae score	mean	1hr	ca. 1	1	fully reversible within: 24 hrs	
conjunctivae score	mean	24, 48, 72hrs	ca. 0	0		

Any other information on results incl. tables

The abnormality was not seen in all rabbits. The conjunctival red flare (rated 1 where the severest rate is 4) and the hyperemia in some blood vessels were seen in three rabbits after one hour from instillation. However these disappeared in 24 hours after the instillation. Muddiness of the cornea, abnormality and conjunctival edema of the iris were not observed to neither rabbit.

Table 1 -1 A Primary Eye Irritation Study of Carbon nanotube in Rabbits (Observation of Eyes)
Gross observation - Scores of ocular irritation, Grading of ocular lesions (OECD TG 405 (2002))

Animal No.	Parameter	At animal selection		Post-dosing							
				1 h		24 h		48 h		72 h	
		R	L	R	L	R	L	R	L	R	L
ET1M01	Cornea, l Opacity: degree of density	0	0	0	0	0	0	0	0	0	0
	Conjunctivae, Redness	0	0	0	1	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0
	Chemosis Swelling	0	0	0	0	0	0	0	0	0	0
ET1M02	Cornea Opacity: degree of density	0	0	0	0	0	0	0	0	0	0
	Conjunctivae Redness	0	0	0	1	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0
	Chemosis Swelling	0	0	0	0	0	0	0	0	0	0
ET1M03	Cornea Opacity: degree of density	0	0	0	0	0	0	0	0	0	0
	Conjunctivae Redness	0	0	0	1	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0
	Chemosis Swelling	0	0	0	0	0	0	0	0	0	0
Mean	Cornea Opacity: degree of density	0	0	0	0	0	0	0	0	0	0
	Conjunctivae Redness	0	0	0	1	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0
	Chemosis Swelling	0	0	0	0	0	0	0	0	0	0

R: Right eye (untreated) L: Left eye (Carbon nanotube B)

Evaluations of ocular reactions were scored as follows:

Cornea:

Opacity: degree of density; 0 = No ulceration or opacity, 1 = Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible, 2 = Easily discernible translucent area; details of iris slightly obscured, 3 = Necrotic area; no details of iris visible; size of pupil barely discernible, 4 = Opaque cornea; iris not discernible through the opacity

Conjunctivae:

Redness; 0 =, 1 = Some blood vessels hyperaemic (injected), 2 = Diffuse, crimson colour; individual vessels not easily discernible, 3 = Diffuse beefy red

Iris: 0 =, 1 = Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect),

2 = Hemorrhage, gross destruction, or no reaction to light

Chemosis:

Swelling; 0 =, 1 = Some swelling above norma, 2 = Obvious swelling, with partial eversion of lids,

3 = Swelling, with lids about half closed, 4 = Swelling, with lids more than half closed

**Table 1 -2 A Primary Eye Irritation Study of Carbon nanotube in Rabbits (Observation of Eyes)
Corneal staining - Scores of corneal epithelium damage, McDonald-Shadduck scoring system -**

Animal No.	Parameter	At animal selection		Approximately 72 hours post-dosing	
		R	L	R	L
ET1M01	Fluorescein staining	0	0	0	0
ET1M02	Fluorescein staining	0	0	0	0
ET1M03	Fluorescein staining	0	0	0	0
Mean	Fluorescein staining	0	0	0	0

R: Right eye (untreated) L: Left eye (Carbon nanotube)

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

slightly irritating

Criteria used for interpretation of results

not specified

Conclusions

The abnormality was not seen in all rabbits. The conjunctival red flare (rated 1 where the severest rate is 4) and the hyperemia in some blood vessels were seen in three rabbits after one hour from instillation. However these disappeared in 24 hours after the instillation. Muddiness of the cornea, abnormality and conjunctival edema of the iris were not observed to neither rabbit.

7.4 Sensitisation

7.4.1 Skin sensitisation

Endpoint study record: Baytubes: Skin sensitisation.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
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 MaterialScience	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 406 (Skin Sensitisation)	

Test materials

Test material identity

Identifier	Identity
other:	Baytubes

Test animals

Species

guinea pig

Applicant's summary and conclusion

Conclusions

no sensitizing effect

Endpoint study record: Graphistrength C100: Skin sensitisation.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

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 method**

in vitro

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD TG 429	

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:	Arkema Graphistrength C100

Details on test material

- Name of test material (as cited in study report): Graphistrength C100
- Other: Micronized, the mean diameter of the agglomerates was reduced to 30 µm instead 400 µm to increase the bioavailability

Test animals**Species**

mouse

Strain

CBA

Sex

female

Test system**Traditional sensitisation test****Vehicle**

polyethylene glycol

Concentration

0.25, 0.5, 1.0 and 2.5%

No. of animals per dose

4 animals/dose

Positive control substance(s)yes α -hexylcinnamaldehyde (HCA)**LLNA*****Positive control substance(s)***

hexyl cinnamic aldehyde (CAS No 101-86-0)

Any other information on materials and methods incl. tables

The potential of Graphistrength C100 (micronized, the mean diameter of the agglomerates was reduced to 30 μ m instead 400 μ m to increase the bioavailability) to induce delayed contact hypersensitivity was evaluated using the murine Local Lymph Node Assay (LLNA) (Sire, 2008). Evaluation of local irritation was also carried out in parallel. This study was conducted in compliance with the principles of Good Laboratory Practice Regulations. A preliminary test was first performed in order to define the concentrations of test item to be used in the main test. In the main test, twenty-eight female CBA/J mice were allocated to seven groups:

- Five treated groups of four animals receiving the test item Graphistrength C100 micronized at the concentration of 0.1, 0.25, 0.5, 1 or 2.5%,
- One negative control group of four animals receiving the vehicle (propylene glycol),
- One positive control group of four animals receiving the reference item, α -hexylcinnamaldehyde (HCA), a moderate sensitizer, at the concentration of 25%.

The test item was not soluble in the recommended vehicles, consequently, a homogenous suspension,

obtained in propylene glycol, was used for the study. The maximum practicable concentration in this vehicle was 2.5%. Therefore, the concentrations selected for the preliminary test were 0.25, 0.5, 1 and 2.5%. Since the test item was non-irritant in the preliminary test, the highest concentration retained for the main test was the maximal practicable concentration (2.5%).

Results and discussion

Positive control results

A significant lymphoproliferation was noted in the positive control group given HCA at 25%, the study was therefore considered valid.

LLNA

Any other information on results incl. tables

No mortality and no clinical signs were observed during the study. A black coloration of the skin of the ears was noted on days 2 and 3 in all animals treated at the concentrations of 1 and 2.5% and on day 6 in 2/4 animals treated at the concentration of 2.5%. No noteworthy increase in ear thickness was observed in the animals of the treated groups. No noteworthy lymphoproliferation was noted at any of the tested concentrations. Graphistrength C100 micronized did not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay.

Applicant's summary and conclusion

Conclusions

Graphistrength is not a sensitizer.

Endpoint study record: Mitsui MWNT-7: Skin sensitisation.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 TG406

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ema, M., Matsuda, A., Kobayashi, N., Naya, M. and Nakanishi, J.	2011	Evaluation of deramal and eye irritation and skin sensitization due to carbon nanotubes	Regulatory Toxicology and Pharmacology, 61, 276-281, (2011)	Ina Research				

Materials and methods**Type of method**

in vivo

Type of study

Buehler test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 406 (Skin Sensitisation)	

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): 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: 60 nm

Test animals**Species**

guinea pig

Strain

Hartley

Sex

male

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

- Source: Japan SLC Inc, japan

- Age at study initiation: 5 - 6 weeks old
- Weight at study initiation: 0.33 - 0.41 kg bw
- Housing: Animals were housed individually in standard aluminum cages.
- Diet (e.g. ad libitum): LRC4; Oriental Yeast Co. Ltd., (ad libitum)
- Water (e.g. ad libitum): Tap water (ad libitum)
- Acclimation period: 6 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 19.0 - 25.0 °C
- Humidity (%): 40 - 70%
- Air changes (per hr): 15 - 21 times/hr
- Photoperiod (hrs dark / hrs light): 12 hrs dark/ 12 hrs light

Test system

Traditional sensitisation test

Route of induction exposure

epicutaneous, occlusive

Route of challenge exposure

epicutaneous, occlusive

Vehicle

olive oil

Concentration

2%

No. of animals per dose

10 for negative control 10 for positive control 20 for material treating

Details on study design (Traditional tests)

The test was executed using 40 male Slc: Hartley guinea pigs of 5-6 weeks of age, 10 for negative control, 10 for positive control and 20 for material treated groups, following OECD TG 406. CNTs were prepared at the maximum concentration which allowed a uniform mixture. The specimens are prepared in a same concentration using olive oil and Vaseline as medias for sensitization and elicitation respectively. As for sensitization, the lint (2 x 2.5 cm), on which 0.4g of sensitization specimen was applied, was occlusive-dressed for 6 hours to the skin of the regio lateralis of guinea pig where the pelage had been removed on the day before (day 0). The sensitizations were performed once a week and three times in total to the area where the pelage was removed on the day before (day 7 and day 14). As for elicitation, in two weeks (day 28) after the final sensitization, a lint (2 x 2 cm) on which 0.2g of elicitation specimen was uniformly applied was occlusive-dressed for 6 hours to the pelage removed regio lateralis where the sensitization had not performed. In 24 and 48 hours after elicitation, the skin where the elicitation was applied was observed.

Positive control substance(s)

yes (1% DNCB)

Results and discussion

LLNA

Any other information on results incl. tables

No abnormality in conjunction with the test was observed to the all guinea pigs and no elicitation effect was seen in all observations

Overall remarks, attachments

Attached full study report

Attached full study report
③ M-SWCNT irri-sensitize (Reg.Tox.Pharm.P276-281).pdf / 565.61 KB (application/pdf): Appendix 1

Illustration (picture/graph)

Skin sensitization study of MWNTs in guinea pigs.

Group	Substance for induction	Substance for challenge	No. of guinea pigs	Hours challenge
Test of MWNT-7 Negative control	Saline	White petrolatum	10	24
				48
		2% MWNT-7	10	24
MWNT-7	2% MWNT-7			48
		White petrolatum	20	24
				48
Positive control	1% DNCB	2% MWNT-7	20	24
				48
		Acetone	10	24
				48
		0.1% DNCB	10	24
				48

^a Dermal responses were scored according to Magnusson and Kilgman (1969).

^b Positive ratio = no. of guinea pigs with positive response/no. of guinea pigs with negative response.

Applicant's summary and conclusion

Interpretation of results

not sensitising

Criteria used for interpretation of results

not specified

Conclusions

2% of Mitsui MWnt-7 does not show the skin sensitization effect

7.5 Repeated dose toxicity

7.5.1 Repeated dose toxicity: oral

Endpoint study record: Nanocyl NC7000: Repeated dose toxicity: oral.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
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

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: 28 days repeated dose toxicity Modified OECD TG 420	

Applicant's summary and conclusion

Conclusions

No toxicity observed up to 0.5 mg/kg.day

7.5.2 Repeated dose toxicity: inhalation

Endpoint study record: Baytubes: Repeated dose toxicity: inhalation.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 TG413

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	Pauluhn	2010	Subchronic 13-week Inhalation Exposure of Rats to Multiwalled Carbon Nanotubes: Toxic Effects are determined by Density of Agglomerate Structures, not fibrillar Structures	Toxicol. Sci., 113: 226-242, (2010)					
publication	Pauluhn	2010	Multi-walled carbon nanotubes (BaytubesR): Approach for derivation of occupational exposure limit	Regul Toxicol Pharmacol., 57,78-89, (2010)					

Materials and methods**Test type**

subchronic

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	

Test materials**Test material identity**

Identifier	Identity
other:	Baytubes

Test animals**Species**

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Winkelmann, Borcheln, Germany
- Age at study initiation:
- Weight at study initiation:
- Fasting period before study:
- Housing: Rats were singly housed in polycarbonate cages containing low dust wood shavings as bedding material.
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 2 weeks

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22°C
- Humidity (%): 40-60%
- Photoperiod (hrs dark / hrs light): 12hrs dark/12hrs light

Administration / exposure

Route of administration

inhalation

Type of inhalation exposure

nose only

Duration of treatment / exposure

13 consecutive weeks

Frequency of treatment

6 hrs/day, 5day/week

Doses/concentrations

0, 0.1, 0.4, 1.5, and 6 mg/m³

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes

- Time schedule: Before and after each exposure

DETAILED CLINICAL OBSERVATIONS: No data

BODY WEIGHT: Yes

- Time schedule for examinations: Twice weekly on Mondays and Fridays during the exposure period and once weekly during the postexposure period.

FOOD CONSUMPTION: Yes

- Time schedule for examinations: Once per week during the exposure period

WATER CONSUMPTION: No data

OPHTHALMOSCOPIC EXAMINATION: Yes

- Time schedule for examinations: Prior to start and at the end of the exposure period.

HAEMATOLOGY: Yes

- Time schedule for collection of blood: At the end of the 13-week study period

- Anaesthetic used for blood collection: No data

- Animals fasted: No data- How many animals: 10 rats per group per sex.

- Parameters checked in table [No.?] were examined. The parameters determined were compliant with those defined by OECD#413 (OECD 2008)

CLINICAL CHEMISTRY: Yes

- Time schedule for collection of blood: At the end of the 13-week study period

- Animals fasted: No data

- How many animals: 10 rats per group per sex.

- Parameters checked in table [No.?] were examined. The parameters determined were compliant with those defined by OECD#413 (OECD 2008)

URINALYSIS: Yes

- Time schedule for collection of urine: At the end of the 13-week study period

- Metabolism cages used for collection of urine: No data

- Animals fasted: No data

- How many animals: 10 rats per group per sex.

- Parameters checked in table [No.?] were examined. The parameters determined were compliant with those defined by OECD#413 (OECD 2008)

NEUROBEHAVIOURAL EXAMINATION: Yes

- Time schedule for examinations: No data

- Dose groups that were examined: No data

Sacrifice and pathology

GROSS PATHOLOGY: Yes

Organs weighed: Adrenal glands, brain, heart, kidneys, liver, lung, lung-associated lymph nodes (LALNs), ovaries, spleen, testes, and thymus

HISTOPATHOLOGY: Yes

Organs examined: Light microscopy examined (entire respiratory tract and LALN: all groups; extrapulmonary organs: control and high group): adrenals, aorta, bone (femur), including joint and bone marrow (in femur and sternum and bone marrow smear), brain (cerebrum, cerebellum, and “medulla oblongata”), coagulating gland, epididymides (including accessory glands), esophagus, eyes (including eyelids), Harderian gland, head (pharynx and nasal cavities according to Young, 1981), heart, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, kidneys with pelvis, lacrimal glands (extraorbital glands), larynx, liver, lungs, lymph nodes (hilar region, cervical, mandibular, mesenteric, and popliteal), mammary gland, muscles (biceps and quadriceps), ovaries, pancreas, parathyroids, pituitary, prostate, salivary glands (head), sciatic nerve, seminal vesicles, skin (flank and muzzle/facial area), spinal cord (cervical, thoracic, and lumbar), spleen, sternum, testes, thymus, thyroid, tongue, trachea, urinary bladder, ureters, urethra, uterus (including cervix), vagina, and zymal glands.

Statistics

Body weights, BAL data, organ weights, food and water consumption, hematology, clinical pathology, and urinalysis data were compared using one-way ANOVA and Dunnett or Tukey-Kramer post hoc tests. Histopathological findings were compared with the concurrent control using Fisher's Exact Test. For all tests, the criterion for statistical significance was set at $p < 0.05$. Asterisks in figures and tables denote statistically significant differences to the concurrent air control group, * $p < 0.05$ and ** $p < 0.01$.

Results and discussions**Effect levels**

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	ca. 0.1 mg/m ³ air			

Results of examinations***Clinical signs and mortality***

no effects

Body weight and weight gain

no effects

Food consumption

no effects

Food efficiency

no data

Water consumption

no effects

Ophthalmoscopic examination

no effects

Haematology

no effects

Clinical chemistry

no effects

Urinalysis

no effects

Neurobehaviour

no data

Organ weights

yes

Gross pathology

no data

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

not examined

Details on results**- ORGAN WEIGHTS:**

0.4 mg/m³: Lung and LALN weights were significantly increased.

- HISTOPATHOLOGY: NON-NEOPLASTIC

Histopathology revealed principal exposure-related lesions at 0.4 mg/m³ and above in the upper respiratory tract (goblet cell hyper- and/or metaplasia, eosinophilic globules, and focal turbinate remodeling) and the lower respiratory tract (inflammatory changes in the bronchioalveolar region and increased interstitial collagen staining). Granulomatous changes and a time-dependent increase of a bronchioalveolar hyperplasia occurred at 6 mg/m³.

-

OTHER FINDINGS

- MWCNT Disposition in Lungs and LALNs Kinetic analyses demonstrated a markedly delayed clearance of MWCNT from lungs at overload conditions. Translocation into LALNs occurred at 1.5 and 6 mg/m³ and required at least 13 weeks of study to become detectable.

- BRONCHOALVEOLAR

Lavage Sustained elevations in BAL polymorphonuclear neutrophils and soluble collagen occurred at these concentrations with borderline effects at 0.4 mg/m³.

Any other information on results incl. tables

The subchronic exposure to respirable solid aerosols of MWCNT was tolerated without effects suggestive of systemic toxicity. Kinetic analyses demonstrated a markedly delayed clearance of MWCNT from lungs at overload conditions. Translocation into LALNs occurred at 1.5 and 6 mg/m³ and required at least 13 weeks of study to become detectable. At these exposure levels the lung and LALN weights were significantly increased. Sustained elevations in BAL PMNs and soluble collagen occurred at these concentrations with borderline effects at 0.4 mg/m³. Histopathology revealed principal exposure-related lesions at 0.4 mg/m³ and above in the upper respiratory tract (goblet cell hyper- and/or metaplasia, eosinophilic globules, focal turbinate remodeling) and the lower respiratory tract (inflammatory changes in the bronchio-alveolar region, increased interstitial collagen staining). Granulomatous changes and a time-dependent increase of a bronchio-alveolar hyperplasia occurred at 6 mg/m³. All endpoints examined were unremarkable at 0.1 mg/m³ (NOAEL).

Applicant's summary and conclusion

Conclusions

In summary, this study demonstrates that the induced pathological changes are consistent with overload-related phenomena. Hence, the etiopathological sequence of inflammatory events caused by this type of MWCNT appears to be related to the high displacement volume of the low-density MWCNT-assemblage structure rather than to any yet ill-defined intrinsic toxic property. Thus, the hypothesis of study is verified, namely common denominators between carbon black and MWCNT do exist.

Endpoint study record: Graphistrength C100: Repeated dose toxicity: inhalation.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

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

Test guideline

Qualifier	Guideline	Deviations
no guideline followed	other guideline: 5-day range finding study	

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 animals**Species**

rat

Strain

Wistar

Sex

male/female

Administration / exposure**Route of administration**

inhalation

Type of inhalation exposure

nose only

Details on inhalation exposure

A respirable dust aerosol was generated using a SAG 410 Solid Aerosol Generator connected to a micronizing jet mill and a cyclone and an elutriator thereafter.

Duration of treatment / exposure

6 hrs/d x 5 d

Doses/concentrations0.066, 0.26 and 1.30 mg/m³**Basis** analytical conc.**MMAD / GSD**

The particles of the aerosol were considered to be respirable for rats, with gravimetrically determined Mass Median Aerodynamic Diameters (MMAD) of 1.93 - 2.56, 1.97 and 1.84 - 1.85 µm and Geometric Standard Deviations (GSD) of 1.30 - 4.33, 1.94 and 1.76 - 1.93, respectively. Mean percentages of particles lower than 3 µm were 68.4, 73.6 and 78.3%, respectively.

No. of animals per sex per dose

20 males and 10 females per group

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes

DETAILED CLINICAL OBSERVATIONS: No data

BODY WEIGHT: Yes

FOOD CONSUMPTION:

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

FOOD EFFICIENCY:

- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No data

WATER CONSUMPTION: No

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes

- Time schedule for collection of blood: On the day of sacrificed (24 hours after at the last exposure and after a 4-week treatment-free period)

- Anaesthetic used for blood collection: No data

- Animals fasted: No data

- How many animals: 5 males

- Parameters examined: cell count, viability and defferential cell counts

CLINICAL CHEMISTRY: Yes

- Time schedule for collection of blood: On the day of sacrificed (24 hours after at the last exposure and after a 4-week treatment-free period)

- Animals fasted: No data

- How many animals: 5 males

- Parameters examined: LDH, ALP, GGT and proteins

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No

OTHER:

Sacrifice and pathology

GROSS PATHOLOGY: Yes

HISTOPATHOLOGY: Yes

Other examinations

Broncho-alveolar lavage (BAL) fluids

Results and discussions

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption

no effects

Food efficiency

no data

Water consumption

not examined

Ophthalmoscopic examination

not examined

Haematology

yes

Clinical chemistry

yes

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

no effects

Gross pathology

no effects

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

not examined

Details on results**HAEMATOLOGY**

Slight increase in neutrophil count was observed at 1.30 mg/m³ (2.8% vs. 0.3% in controls) after the 5-day treatment, which disappeared after the 4-week recovery period.

CLINICAL CHEMISTRY

Levels were statistically significantly increased at 1.30 mg/m³ (11.4 vs. 3.2 U/L) but were normal after the recovery and protein values were statistically significantly increased at 0.26 and 1.30 mg/m³ after exposure (0.95 and 0.83 vs. 0.04 g/L) and after the 4-week recovery period (0.64 and 0.75 vs. 0.21 g/L).

HISTOPATHOLOGY: NON-NEOPLASTIC

Treatment-related microscopic findings were noted in the lungs of male and female rats, both after the 5-day exposure and the recovery period. Dose-dependent increased black inclusions were observed in the cytoplasm of infiltrated macrophages, indicating an adequate exposure of the lungs. Macrophage infiltration of the lung (grade 2) was observed at 1.30 mg/m³ in 3 males and 3 females after the 5-day exposure and in 4 males and 4 females after the recovery period. Hypertrophy of the bronchial and bronchiolar cells was observed at 1.30 mg/m³ in 4 males (3 grade 1 and 1 grade 2) and 2 females (1 grade 1 and 1 grade 2) after the 5-day exposure and in 2 males and 2 females (all grade 1) after the recovery period. No treatment-related microscopic findings were observed in the other organs examined.

Endpoint study record: Mitsui MWNT-7: Repeated dose toxicity: inhalation.002**Administrative Data**

Purpose flag supporting study
Study result type experimental result
Reliability 4 (not assignable)
Rationale for reliability incl. deficiencies Secondary source

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Japan Bioassay Research Center				will be published in 2012					

Materials and methods**Test type**

subchronic

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	

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:	Hodogaya (Mitsui) MWNT-7

Test animals**Species**

rat

Strain

Fischer 344

Sex

male/female

Administration / exposure**Route of administration**

inhalation

Type of inhalation exposure

whole body

Duration of treatment / exposure

6 hrs/d x 5 d/w x 13 w

Doses/concentrations0.2, 1.0, 5.0 mg/m³**Basis** nominal conc.**Details on study design**

F344 rats of both sexes were exposed by inhalation to MWCNT aerosol using the whole-body exposure system (The spin air sieve system, Japan Bioassay Research Center) for 13 weeks (6 hrs/day, 5 days/week)

at concentrations of 0.2, 1 or 5 mg/m³.

Results and discussions

Results of examinations

Any other information on results incl. tables

MWCNTs were deposited in the lungs of all exposed groups. Lung weight significantly increased in 1 and 5 mg/m³MWCNT-exposed rats. Histopathologically, granulomatous changes were found in the lung of all MWCNT-exposed groups, and localized fibrosis of the alveolar wall was also seen in the lung of 1 and 5 mg/m³ MWCNT-exposed rats. In bronchoalveolar lavage analysis, acute inflammatory changes were observed in all MWCNT-exposed groups in concentration-dependent manner.

Thus, even low concentration exposure of MWCNT induced the granulomatous changes, and histopathological changes were aggravated compared with the change observed in our previous study by 2 weeks inhalation exposure.

Endpoint study record: Mitsui MWNT-7: Repeated dose toxicity: inhalation.003

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	Porter, DW., Hubbs, AF., Chen, BT., McKinney, W., Mercer, RR., Wolfarth, MG., Battelli, L., Wu, N., Sriram, K., Leonard, S., Andrew, M., Willard, P., Tsuruoka, S., Endo, M., Tsukada, T., Munekane, F., Frazer, DG. and Castranova, V.	2012	Acute pulmonary dose-responses to inhaled multi-walled carbon nanotubes.	Nanotoxicology. 2012 Sep 13. [Epub ahead of print]					

Data access

data published

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

GLP compliance

no data

Test materials**Test material identity**

Identifier	Identity
other:	Hodogaya (Mitsui) MWNT-7

Details on test material

The distribution had a mass median aerodynamic diameter (MMAD) of 1.5 µm and geometric standard deviations (GSD) of 1.67 µm. Mass mode Aerodynamic Diameter equaled 1.3 µm and a Count Mode Aerodynamic Diameter of 0.42µm. A range of single fiber like nanotubes to tangled agglomerates. Impurities: Trace metal contamination was 1.32% (with iron being the major contaminant at 1.06%). MWCNTs were produced by Catalytic Chemical Vapor Deposition (CVD) and floating reactant methods followed by high temperature treatment

- Name of test material (as cited in study report): MWNT-7 (Hodogaya Chemical Company)
- Lot/batch No.: #061220-31

Test animals**Species**

mouse

Strain

C57BL

Sex

male

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

- Source: Jackson Laboratories (Bar Harbor, ME)
- Age at study initiation: 7 weeks old
- Housing: Animals were individually housed in polycarbonate isolator ventilated cage, which were provided HEPA-filtered air. Autoclaved Alpha-Dri virgin cellulose chips and hardwood Beta-chips were used as bedding.
- Diet (e.g. ad libitum): Harlan Teklad Rodent Diet 7913 (ad libitum)
- Water (e.g. ad libitum): Tap water (ad libitum)
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS

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

Administration / exposure**Route of administration**

inhalation

Type of inhalation exposure

whole body

Vehicle

no data

Duration of treatment / exposure

2, 4, 8 or 12day

Frequency of treatment

5 hrs/d

Doses/concentrations

10mg/m³

Basis nominal conc.

MMAD / GSD

Mass median aerodynamic diameter (MMAD): 1.5 µm

Geometric standard deviations (GSD): 1.67 µm. Mass mode Aerodynamic Diameter equaled 1.3 µm and a Count Mode Aerodynamic Diameter of 0.42 µm. A range of single fiber like nanotubes to tangled agglomerates. Impurities: Trace metal contamination was 1.32% (with iron being the major contaminant at 1.06%). MWCNTs were produced by Catalytic Chemical Vapor Deposition (CVD) and floating reactant methods followed by high temperature treatment in argon at 2500! C using a continuousfurnace (Kim et al. 2005).

Control animals

yes

Details on study design

Mice were exposed to 10 mg/m³, 5 hours/day, for 2, 4, 8, or 12 days. Mice sacrificed 24 hours after the end of inhalation exposure for 2-12 days. Lung burden was determined chemically, using the method of Elder et al., 2005. Bronchoalveolar lavage samples were used to measure cytotoxicity (fluid LDH activity), air/blood barrier damage (fluid albumin levels) and cell differentials. Alveolitis, fibrosis and bronchiolar epithelial hyperplasia of histological samples of lung tissue were scored by a board-certified pathologist.

Examinations***Sacrifice and pathology***

HISTOPATHOLOGY: Yes

Histological changes in the nose, lung and trachea-bronchial lymph node were assessed.

Other examinations

Pulmonary inflammation, damage and fibrosis were also assessed at one-day post-exposure. In addition, lung burden was assessed.

- MWCNT lung burden
- Whole lung lavage (WLL) fluid LDH, albumin and cytokine measurement

Statistics

All outcomes yielding quantitative values were analysed with parametric analysis of variance (ANOVA). This was performed using an unequal variance model available in SAS PROC MIXED. Ordinal measurements resulting from histopathology were analysed with non-parametric ANOVA. This was performed using the SAS procedure PROC NPAR1WAY with an exact Kruskal-Wallis test to account for ties in the data. All statistical tests in this study are two-tailed with an alpha = 0.05 significance level.

Results and discussions**Results of examinations*****Clinical signs and mortality***

no data

Body weight and weight gain

no data

Food consumption

not examined

Food efficiency

not examined

Water consumption

not examined

Ophthalmoscopic examination

not examined

Haematology

not examined

Clinical chemistry

not examined

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

not examined

Gross pathology

no data

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

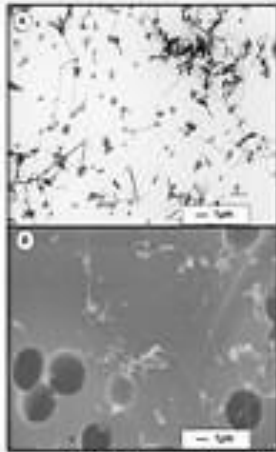
not examined

Details on results

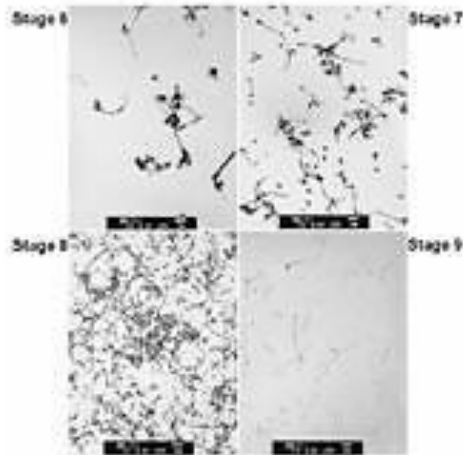
- MWCNT lung burdenMWCNT lung burden was linearly related to exposure duration.
- Whole lung lavage (WLL) fluid LDH, albumin and cytokine measurementThese parameters significantly increased in MWCNT-exposed mice versus controls and were dose-dependent.
- Histopathological examinationHistopathologic alterations identified in the lung included (1) bronciolocentric inflammation, (2) bronchiolar epithelial hyperplasia and hypertrophy, (3) fibrosis, (4) vascular changes and (5) rare pleural penetration. MWCNT translocated to the lymph node where the deep paracortex was expanded after 8 or 12 days.

Overall remarks, attachments

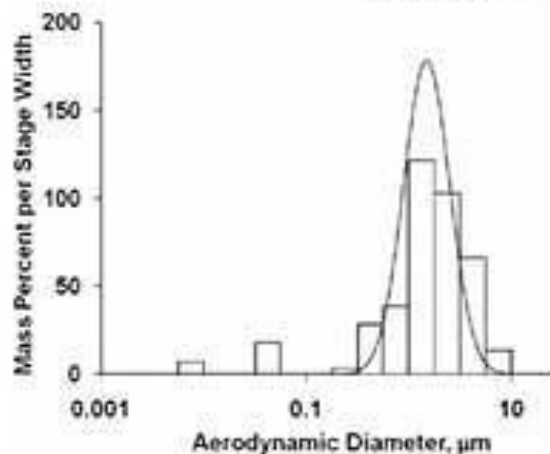
Illustration (picture/graph)



TEM and field emission SEM images of MWCNT aerosol samples.



Transmission electron microscopy images of different stages in a MOUDI cascade



A typical size distribution of the MWCNT aerosol in the exposure chamber (M=mass concentration and D_{ac} =aerodynamic diameter). The distribution has a MMAD=1.5 μm and a GSD of 1.67.

Applicant's summary and conclusion

Conclusions

Acute inhalation of MWCNT induced dose-dependent pulmonary inflammation and damage with rapid development of pulmonary fibrosis, and also demonstrated that MWCNT can reach the pleura after inhalation exposure.

Executive summary

This study investigated the in vivo pulmonary toxicity of inhaled multi-walled carbon nanotubes (MWCNT). Mice-inhaled aerosolized MWCNT (10 mg/m³, 5 h/day) for 2, 4, 8 or 12 days. MWCNT lung burden was linearly related to exposure duration. MWCNT-induced pulmonary inflammation was assessed by determining whole lung lavage (WLL) polymorphonuclear leukocytes (PMN). Lung cytotoxicity was assessed by WLL fluid LDH activities. WLL fluid albumin concentrations were determined as a marker of alveolar air-blood barrier integrity. These parameters significantly increased in MWCNT-exposed mice versus controls and were dose-dependent. Histopathologic alterations identified in the lung included (1) bronchiolocentric inflammation, (2) bronchiolar epithelial hyperplasia and hypertrophy, (3) fibrosis, (4) vascular changes and (5) rare pleural penetration. MWCNT translocated to the lymph node where the deep paracortex was expanded after 8 or 12 days. Acute inhalation of MWCNT induced dose-dependent pulmonary inflammation and damage with rapid development of pulmonary fibrosis, and also demonstrated that MWCNT can reach the pleura after inhalation exposure.

Endpoint study record: Hodogaya (Mitsui) MWNT-7 Repeated dose toxicity: inhalation.004

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Study was done under GLP following OECD TG

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Tatsuya Kasai, Yumi Umeda, Makoto Ohnishi, Hitomi Kondo, Tetsuya Takeuchi, Shigetoshi Aiso, Tomoshi Nishizawa, Michiharu Matsumoto, and Shoji Fukushima	2014	Thirteen-week study of toxicity of fiber-like multi-walled carbon nanotubes with whole-body inhalation exposure in rats	Nanotoxicology					

Data access

other: The paper will be soon published

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	

GLP compliance

yes

Test materials

Test material identity

Identifier	Identity
other:	Hodogaya (Mitsui) MWNT-7

Details on test material

Fiber-like straight MWCNTs were purchased from Hodogaya Chemical Co., Ltd. (MWNT-7, Lot No. 071223, 080126, Tokyo) and were used in the present study as produced, i.e. without further purification or sieving. Carbon purity of >99.6% (No. 071223) and >99.8% (No. 080126). A nominal mean diameter is 40–90 nm, an aspect ratio is >100 and the surface area is 24–28 m²/g by supplier's inspection sheet. The actual diameter and length were measured before the experiments. The mean diameter and length were 90.7 nm and 5.7 μm, respectively. The lengths of 48.7 % of tubes were greater than 5 μm.

Test animals

Species

rat

Strain

other: F344/DuCrIjCrIj

Sex

male/female

Details on test animals and environmental conditions

F344/DuCrIjCrIj rats of both sexes were purchased from Charles River Japan Inc. (Kanagawa, Japan) at 4 weeks of age. The animals were quarantined and acclimated for 2 weeks before the onset of experimentation.

Administration / exposure

Route of administration

inhalation: aerosol

Type of inhalation exposure

whole body

Vehicle

clean air

Details on inhalation exposure

The aerosol generation method (cyclone sieve method) of Kasai et al. (2013) was used to generate MWCNT aerosols. Briefly, the aerosol generator dry-aerosolizes MWCNT with an upward spiraling airstream, classifies particles by gravitational and centrifugal forces, limits particle size with a sieve and provides the inhalation chamber with size-limited MWCNT aerosols.

Details on analytical verification of doses or concentrations

The particle number concentrations of MWCNT in the chambers were monitored in real time with an optical particle controller (OPC: OPC-AP-600, Sibata Scientific Technology, Ltd., Tokyo, Japan), and chamber MWCNT concentrations were held constant by controlling the movement of dust feeder with a feedback loop using the OPC. This latter is used to continuously monitor particle concentrations (counts per minute: CPM) of MWCNT aerosols detected as particles $>0.3 \mu\text{m}$, and sampling flow rate is 2.8 l/min in the inhalation chamber. During the first exposure period, OPC was set to count 110 000, 550 000 and 2 900 000 particles per minute (CPM) for 0.2, 1 and 5 mg/m³ exposures, respectively.

Duration of treatment / exposure

For 13 weeks.

Frequency of treatment

6 h/day, 5 days/week,

Doses/concentrations

0.20±0.02 mg/m³

Basis analytical conc.

1.01±0.11 mg/m³

Basis analytical conc.

5.02±0.25 mg/m³

Basis analytical conc.

MMAD / GSD

1.4 μm -1.6 μm

No. of animals per sex per dose

10

Control animals

yes, concurrent no treatment

Details on study design

Clinical observations, urinary examination and hematological and blood biochemical analysis, cytological and biochemical analyses of bronchoalveolar lavage fluid, organ weights and pathological examination were performed during and after exposure period.

Examinations

Statistics

Organ weights and biochemical and cytological parameters in the BALF were analyzed by Dunnett's multiple comparison test. The chi-squared test was employed to evaluate the statistical significance of the differences in the incidences of histopathological lesions between MWCNT-exposed and clean air-exposed groups. Values of $p < 0.05$ were considered significant.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
LOAEL	0.2 mg/m ³ air	test mat.	male/female	

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Haematology

no effects

Clinical chemistry

no effects

Urinalysis

no effects

Any other information on results incl. tables

Group (mg/m3)	Male				Female			
	0	0.2	1	5	0	0.2	1	5
Number of animals examined	10	10	10	10	10	10	10	10
Histopathological findings								
Nasal cavity								
Goblet cell hyperplasia	0	0	10**(1.0)	10**(1.6)	0	0	10**(1.0)	10**(1.8)
Eosinophilic globules, olfactory epithelium	1(1.0)	2(1.0)	10**(1.1)	10**(1.9)	1(1.0)	1(1.0)	10**(1.0)	10**(2.0)
Eosinophilic globules, respiratory epithelium	1(1.0)	2(1.0)	10**(1.0)	10**(1.0)	0	0	10**(1.0)	10**(1.0)
Nasopharynx								
Goblet cell hyperplasia	0	1(1.0)	10**(1.0)	10**(1.0)	0	0	10**(1.0)	10**(1.4)
Lung								
Granulomatous change	0	1(1.0)	8**(1.0)	10**(1.4)	0	0	4(1.0)	10**(1.1)
Focal fibrosis	0	0	10**(1.0)	10**(1.4)	0	0	9**(1.0)	10**(1.1)
Visceral pleura								
Inflammatory infiltration	0	0	0	1(0)	0	0	0	1(1.0)
Deposition of MWCNT								
Nasal cavity, respiratory epithelium	0	1	7**	10**	0	4	7**	10**
Larynx, epithelium	0	3	4	5*	0	2	3	6*
Trachea, epithelium	0	8**	10**	10**	0	6*	7**	7**
Lung								
Bronchiolar space	0	10**	10**	10**	0	10**	10**	10**
Alveolar space	0	10**	10**	10**	0	10**	10**	10**
Alveolar wall	0	4	10**	10**	0	3	10**	10**
BALT	0	7**	10**	10**	0	5*	8**	8**
MLN	0	3	10**	9**	0	3	6**	10**

Values indicate number of animals being the lesion.
The value in parenthesis indicate the average of severity grade index of the lesion in affected animals.
The average of severity grade index are calculated with the following equation.

$$[\Sigma(\text{grade} \times \text{number of animals with grade}) \div \text{number of affected animals}]$$

Grade: 1 = slight, 2 = moderate, 3 = marked, 4 = severe.
Significant difference; *p≤ 0.05, **p≤ 0.01 by chi-squared test

Overall remarks, attachments

Illustration (picture/graph)

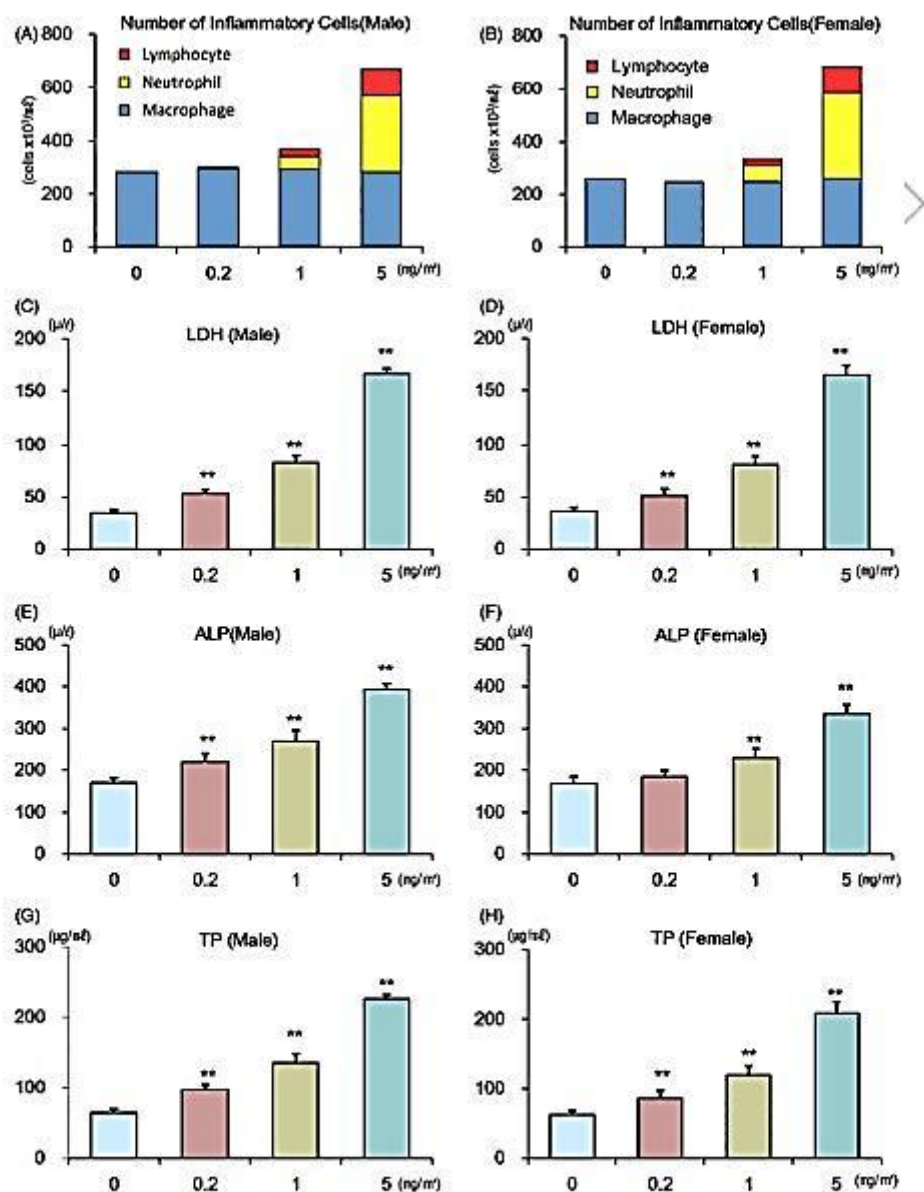


Figure 2. Cytological and biochemical analyses of bronchoalveolar lavage fluid (BALF). Cytological analyses (A and B): A total of >500 BALF inflammatory cells, including neutrophils, lymphocytes and alveolar macrophages, were counted for each group under a light microscope. Finally, corrected for total cells per 1 ml in the BALF. (A) Male and (B) Female. Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities and total protein (Tp). (C-H) Lactate dehydrogenase (LDH) activities (C and D), alkaline phosphatase (ALP) activities (E and F) and total protein (Tp) concentrations (G and H) in the BALF were determined for male and female rats. Error bars indicate the SD for 10 rats. * $p < 0.05$ and ** $p < 0.01$ by Dunnett's multiple comparison test.

Applicant's summary and conclusion

Conclusions

The toxicological data obtained here indicate that fibrous type MWCNT exposure concentration-dependently induced subchronic toxicity effects, such as granulomatous change, focal fibrosis and alteration in BALF parameters. A few MWCNTs were observed in the subpleural area and diaphragm. Moreover, the lung burden of MWCNTs demonstrated that incidences and severity of toxicity depended on exposure concentration, duration and retention. The lowest-observed-adverse-effect level (LOAEL) was estimated to be 0.2 mg/m³ with the endpoint of granulomatous changes and BALF parameters.

Endpoint study record: Nanocyl NC7000: Repeated dose toxicity: inhalation.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 TG413

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						
publication	Ma-Hock L, Treumann S, Strauss V, Brill S, Luiz F, Mertler M, Wiench K, Gamer AO, van Ravenzwaay B, Landsiedel R.	2009	Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months	Toxicol Sci., 112:468-481, (2009)					

Data access

data published

Materials and methods**Test type**

subchronic

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	

Test materials**Test material identity**

Identifier	Identity
other:	Nanocyl NC7000

Details on test material

- Name of test material (as cited in study report): Nanocyl NC 7000
- Analytical purity: 90%
- Impurities (identity and concentrations): 10% metal oxide, of which 9.6% was aluminum oxide with traces of iron and cobalt.
- Other: Diameters: 5 - 15 nm, Length: 0.1 - 10 µm. The specific surface area (Brunauer Emmet Teller method): 250 - 300 m²/g

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Charles River, Germany
- Age at study initiation: 8-9 weeks old
- Housing: When not exposed they were housed three to five per sex per cage in 0.2-m² polycarbonate cages with dust-free bedding and wooden gnawing block.
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period:

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20 - 24°C
- Humidity (%): 30-70%
- Photoperiod (hrs dark / hrs light): 12hrs dark/12hrs light

Administration / exposure

Route of administration

inhalation

Type of inhalation exposure

nose/head only

Duration of treatment / exposure

13 weeks, total 65 exposures

Frequency of treatment

6 hours/day, 5 days/week

Doses/concentrations

0 (control), 0.1, 0.5 or 2.5 mg/m³

No. of animals per sex per dose

10 animals/sex/dose

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes

- Time schedule: At least once daily (three times on exposure days)

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule for examinations: Prior to and weekly during the exposure period

BODY WEIGHT: Yes

- Time schedule for examinations: Weekly

FOOD CONSUMPTION: Yes

- Time schedule for examinations: Weekly

WATER CONSUMPTION: No

OPHTHALMOSCOPIC EXAMINATION: Yes

- Time schedule for examinations: Before (day 6) and at the end of the exposure period (day 84).

HAEMATOLOGY: Yes

- Time schedule for collection of blood: At the end of exposure

- Anaesthetic used for blood collection: Yes (isoflurane) –

Animals fasted: Yes

- How many animals: All animals

- Parameters checked in table [No.?] were examined. white blood cell, red blood cell, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood cell counts

CLINICAL CHEMISTRY: Yes

- Time schedule for collection of blood: At the end of exposure

- Animals fasted: Yes

- How many animals: All animals

- Parameters checked in table [No.?] were examined. alanine and aspartate aminotransferase, alkaline phosphatase and gamma-glutamyltransferase activity, sodium, potassium, chloride, phosphate, calcium, magnesium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides and cholesterol levels, and prothrombin time

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: Yes

- Time schedule for examinations: Tests were performed towards the end of the exposure.

- Dose groups that were examined: First 5 males and 5 females in each group

- Battery of functions tested: sensory activity / grip strength / motor activity:

Sacrifice and pathology

GROSS PATHOLOGY: Yes

HISTOPATHOLOGY: Yes

Statistics

Data are reported as mean $\pm$ standard deviation. Dunnett's test (Dunnett, 1955, 1964) was used for simultaneous comparison of all concentration groups with the control group for food consumption (g/animal), body weights and body weight changes. Kruskal-Wallis (two-sided) tests, followed if significant by pairwise Wilcoxon tests (Hettmansperger, 1984; Miller, 1981; Nijenhuis and Wilf, 1978; Siegel, 1956) were used for clinical chemistry and hematology, functional observation battery and automated motor activity data. Statistical significance was defined as $p < 0.05$ compared with the control group.

Results and discussions

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption

no effects

Food efficiency

no data

Water consumption

not examined

Ophthalmoscopic examination

no effects

Haematology

yes

Clinical chemistry

no effects

Urinalysis

not examined

Neurobehaviour

no effects

Organ weights

yes

Gross pathology

yes

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY

No effects were observed.

BODY WEIGHT AND WEIGHT GAIN

No effects were observed.

FOOD CONSUMPTION

No effects were observed.

OPHTHALMOSCOPIC EXAMINATION

No effects were observed.

HAEMATOLOGY

At the end of the exposure, total white blood cell counts were significantly increased in males and females exposed to 2.5 mg/m³ MWCNT. These increases were due to raised neutrophil counts (relative and absolute) and correspondingly decreased relative lymphocyte counts, indicating slight systemic inflammation.

CLINICAL CHEMISTRY

No effects were observed.

NEUROBEHAVIOUR

No effects were observed.

ORGAN WEIGHTS

Lung weights were increased concentration dependently.

GROSS PATHOLOGY

All animals exposed to the high and mid-concentration had diffuse grey discolored lungs, presumed due to deposition and accumulation of test substance. In addition, discoloration of the mediastinal lymph node was seen in all females and one male exposed to the high concentration as well as in one female and one male exposed to the mid-concentration. Macroscopic lung foci were seen at the high concentration in three

males and three females; these were attributed to macrophage accumulation and/or lipoproteinosis.

HISTOPATHOLOGY: NON-NEOPLASTIC (Tables, 1, 2, 3, and 4)

Histopathology revealed treatment-related lesions in lungs, draining mediastinal lymph nodes, nasal cavity, and larynx and trachea.

Any other information on results incl. tables

Table 1. Effects in lung

	grade	Male			Femlae				
		contro l	0.1 mg/m ³	0.5 mg/m ³	2.5 mg/m ³	contro l	0.1 mg/m ³	0.5 mg/m ³	2.5 mg/m ³
Number of animals		10	10	10	10	10	10	10	10
Relative lung weight		100%	101%	123%*	181%**	100%	104%	134%**	190%**
Diffuse histiocytosis	1	—	8	—	—	—	10	—	—
	2	—	—	9	1	—	—	10	—
	3	—	—	1	9	—	—	—	8
	4	—	—	—	—	—	—	—	2
Granulomatous inflammation	1	—	1	—	—	—	4	3	—
	2	—	—	10	—	—	—	7	1
	3	—	—	—	10	—	—	—	9
Intra-alveolar lipoproteinosis	1	—	—	4	—	—	—	1	—
	2	—	—	3	7	—	—	8	—
	3	—	—	—	3	—	—	1	7
	4	—	—	—	—	—	—	—	3
Diffuse neutrophilic inflammation	1	—	—	10	10	—	—	10	10

Severity grade: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked

*, P<0.05, **: P<0.01 versus control

Table 2. Effects in mediastinal lymph nodes

	grade	Male				Femlae			
		contro l	0.1 mg/m ³	0.5 mg/m ³	2.5 mg/ m ³	control	0.1 mg/m ³	0.5 mg/m ³	2.5 mg/m ³
Number of animals		10	10	10	10	10	10	10	10
Lymphoreticocellular hyperplasia	1	—	—	1	2	—	—	—	1
	2	—	—	1	6	—	—	—	6
	3	—	—	—	—	—	—	—	3
Granulomatous inflammation	1	—	2	4	6	—	4	1	—
	2	—	—	1	4	—	1	8	10
Particles in macrophages		—	5	9	10	—	9	10	10

Severity grade: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Table 3. Effects in nasal cavity levels II-IV

		grade	Male				Femlae			
			control	0.1 mg/m ³	0.5 mg/ m ³	2.5 mg/m ³	control	0.1 mg/ m ³	0.5 mg/m ³	2.5 mg/m ³
Number of animals			10	10	10	10	10	10	10	10
Goblet cell hyperplasia	Level II	1	—	—	3	3	—	—	2	6
		2	—	—	1	6	—	—	—	4
Intraepithelial	Level	1	—	—	—	3	—	—	5	4

eosinophilic inclusions	II	2	—	—	—	—	—	—	—	3
	Level III	1	—	—	7	4	—	—	7	2
		2	—	—	1	5	—	—	—	8
		3	—	—	—	1	—	—	—	—
	Level IV	1	—	—	3	4	—	—	3	6
		2	—	—	—	5	—	—	—	2
Submucosal inflammatory infiltration	Level II	1	—	—	—	—	—	—	—	3
	Level III	1	—	—	3	7	—	—	2	9
	Level IV	1	—	—	—	2	—	—	—	—

Severity grade: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Table 4. Effects in larynx and trachea

		grade	Male				Femlae			
			control	0.1 mg/m ³	0.5 mg/m ³	2.5 mg/m ³	control	0.1 mg/m ³	0.5 mg/m ³	2.5 mg/m ³
Number of animals			10	10	10	10	10	10	10	10
Larynx, focal epithelial flattening	Level I	1	2	1	10	6	3	5	9	9
Larynx, intracellular particles	Level III	1	—	—	1	2	—	—	2	3
Trachea, intracellular particles		1	—	—	1	3	—	—	1	6

Severity grade: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Overall remarks, attachments

Remarks on results including tables and figures

Inhalation exposure to MWCNT produced no systemic toxicity. However, increased lung weights, pronounced multifocal granulomatous inflammation, diffuse histiocytic and neutrophilic inflammation, and intra-alveolar lipoproteinosis were observed in lung and lung-associated lymph nodes at 0.5 and 2.5 mg/m³. These effects were accompanied by slight blood neutrophilia at 2.5 mg/m³. Those effects were concentration-related. At 0.1 mg/m³, there was still minimal granulomatous inflammation in the lung and in lung-associated lymph nodes; a no observed effect concentration was therefore not established in this study.

Endpoint study record: Nikkiso MWCNT: Repeated dose toxicity: oral.001

Administrative Data

Purpose flag	key study
Study result type	experimental result Study period Oct, 2010 to Feb. 2011
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to OECD TG407

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	national Institute of Health Studies		28 days repeated dose of Multi-Walled Carbonnanotubes to rats by oral route		Safety Research Institute for Chemical Compounds Co., Ltd	SR10106			2011-02-27
publication	Mariko Matsumoto (National Institute of Health Sciences) etal.	2012	No toxicological effects on acute and repeated oral gavage doses of single-wall or multi-wall carbon nanotube in rats	J. Toxicol. Sci. Vol.37, No.3, 346-474, 2012	Safety Research Institute for Chemical Compounds Co., Ltd.,				

Materials and methods**Test type**

subchronic

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	

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:	Nikkiso MWCNT

Details on test material

- Name of test material (as cited in study report): Multi-walled carbon nanotube
- Analytical purity: >98%
- Impurities (identity and concentrations): Fe (ca. 20 - 50 ppm); other impurities: unknown
- Lot No.: 04-12/10#1-(4)

- Stability under test conditions: No data.

- Storage condition of test material: room temperature (measured: 21 - 25 °C)

Dose preparation: the high dose and middle dose test solutions were prepared by the following procedures: the test material was accurately weighed and added with the vehicle to obtain the prescribed concentration and mixed. The mixture was filled up to obtain the prescribed volume, and homogenized using an ultrasonic homogenizer (VC-130, Sonics & Materials Inc.) for 5 minutes, and then using a compact ultrasonic cleaning bath (USC-6, Iwaki Glass Co., Ltd.) for 10 minutes. The mixture was homogenized with the ultrasonic homogenizer for 5 minutes. Using a part of the mixture homogenized for 10 minutes by the compact ultrasonic cleaning bath for 10 minutes to prepare the middle dose test solution, the low dose test solution was prepared as follows: a required amount of the mixture was collected and diluted 10 times with the vehicle, and the mixture was homogenized by the ultrasonic homogenizer for 5 minutes. In order not to prevent homogenization, a stirrer was not used. Confirmation of homogeneity and concentrations of the test solutions: The concentrations and the homogeneity of the test solutions were confirmed by the following procedure at the first and final preparation times.

A part of each test sample collected from the upper, middle and lower layers of the test solutions (0.2 mL × 2 tubes for each layer) was sent to the National Institute of Health Sciences at room temperature. In a similar manner, an adequate amount of each test sample was instilled onto the glass slide and microscopically examined (1 slide for each layer) to confirm the homogeneity (dispersion state) of the test material.

Test animals

Species

rat

Strain

other: Crl:CD(SD)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Charles River Laboratories, Japan, Inc.

- Age at study initiation: 5 weeks

- Weight at study initiation: male (158-203 g), female (130-163 g)

- Housing: Animals were individually housed in metallic cages with wire mesh bottoms (260W × 380D × 180H mm)

- Diet: Solid feed (CRF-1; Oriental Yeast Co., Ltd., Japan), ad libitum

- Water: Tap water, ad libitum- Acclimation period: 13 days for males and 14 days for females

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22 ± 3 °C (measured: 20 - 24 °C)

- Humidity (%): 50 ± 20% (measured: 34 - 53%)

- Air changes (per hr): 10-15 time/hour

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

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: 5% Acacia aqueous solution

Analytical verification of doses or concentrations

yes

Duration of treatment / exposure

28 days

Frequency of treatment

1/day

Doses/concentrations

Basis other: 0, 0.5, 5, 50 mg/kg bw/day

No. of animals per sex per dose

12 animals/sex/dose for 0 and 50 mg/kg bw/day (6 animals/sex/dose were used as the recovery group) 6 animals/sex/dose for 0.5 and 5 mg/kg bw/day

Control animals

yes, concurrent vehicle

Details on study design

Rationale for vehicle selection: In the investigation of the vehicles including gum acacia, carmellose sodium, corn oil, which are commonly used in toxicity studies, the test substance showed the best dispersion state in 5% gum acacia.

Rationale for dose selection: In the assay of dispersion state of the test substance, the dose of 2.5 mg/mL was confirmed to be optimum and limit concentration. Ethically, a dosing volume of 20 mL/kg is the limit volume at one time by the oral route. Accordingly, the high dose was set at 50 mg/kg, which is obtained by administration of 2.5 mg/mL at 20 mL/kg, and the middle and low doses were set at 5 and 0.5 mg/kg, respectively, with a common ratio of 10.

-Post-exposure recovery period: 14 days

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes

- Time schedule: All males and females were observed twice per day every day during the administration period and the recovery period.

DETAILED CLINICAL OBSERVATIONS: Yes

BODY WEIGHT: Yes

- Time schedule for examinations: Body weight was measured on days 1, 4, 7, 14, 21 and 28 of administration period, on days 7 and 14 of recovery period.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

- Food consumption: Yes
- Food consumption was measured on days 1, 7, 14, 21 and 28 of administration period, days 7 and 14 of recovery period.

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

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

- Time schedule for collection of blood: Hematological examinations were performed on the blood samples obtained just prior to necropsy.
- Anesthetic used for blood collection: Yes
- Animals fasted: Yes
- How many animals: 6 /sex/dose
- The following were examined: red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (Plat), reticulocyte count (Ret), prothrombin time (PT), activated partial thromboplastin time (APTT) and differential of leukocyte.

CLINICAL CHEMISTRY: Yes

- Time schedule for collection of blood: Clinical chemistry examinations were performed on the blood samples obtained just prior to necropsy.
- Animals fasted: Yes- How many animals: 6/sex/dose
- The following were examined: AST, ALT, ALP, γ -GTP, total cholesterol (T-Cho), triglycerides(TG), glucose (Glu), total protein (TP), Protein fraction, albumin (Alb), A/G ratio (AG), blood urea nitrogen (BUN), creatinine (Crea), total bilirubin (T-Bil), calcium (Ca), inorganic phosphorus (IP), sodium (Na), potassium (K) and chloride (Cl).

URINALYSIS: Yes

- Time schedule for collection of urine: Urinary samples were collected for 3 and 20 hours in the fourth week of the administration period and in the second week of the recovery period.
- The following were examined: pH, Protein, Glucose, Ketone body, Urobilinogen, Bilirubin, Occult blood, Color, Urine Volume, Specific gravity.

NEUROBEHAVIORAL EXAMINATION: Yes

- Time schedule: Once Weekly.

Sacrifice and pathology

GROSS PATHOLOGY: Yes

- Organ weights: Brain, pituitary gland, thyroid gland, parathyroid gland, thymus, adrenal gland, spleen, heart, liver, kidneys, testes, epididymides, prostate, seminal vesicle, ovary, and uterus were weighed.

HISTOPATHOLOGY: Yes

- Examined tissue:[Examinations at the end of administration period]0 and 50 mg/kg group: Brain,

pituitary gland, spinal cord, thymus, , thyroid gland, parathyroid gland, adrenal gland, spleen, heart, tongue, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph node, submandibular lymph node, trachea, lungs, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicle, ovary, uterus, vagina, eyeball, Harderian gland, femur (bone marrow), and sciatic nerve[gross lesions]Ileum diverticulum (male: 5 mg/kg group, No152; female vontrol group, No.305), grandular stomach black patch, mucosa (female: 50 mg/kg recovery group No457), submandibular lymph node swelling (male: 0.5 mg/kg group No205)

Statistics

Continuous data were analyzed by the Bartlett's test for homogeneity of distribution. When homogeneity was recognized, Dunnett's test was conducted for comparison between control and individual treatment groups after one-way layout analysis of variance. If not homogenous, the data were analyzed using the Kruskal-Wallis test followed by Mann-Whitney's U-test.

Any other information on materials and methods incl. tables

Test for oral toxicity was conducted based on OECD TG407. However, the maximum dose of 1000 mg/kg required by the guideline was impracticable because of very high specific volume of MWCNT. Rats (CrI: CD, 6 animals/sex/dose) were administered with the suspensions (5% arabic-gumaqueous solution) at doses of 0, 0.5, 5 and 50 mg/kg/day by gavage. The 2.5 mg/ml of MWCNT is prepared at the maximum concentration allowing a uniform suspension in vehicle. The suspensions of 20 ml /kg bw were dosed once daily by gavage for 28 days. In the control and highest dose group, animals were observed for 14 days after the administration period, as the recovery study. Clinical signs (general, FOB), body weight, food consumption, urinary analysis, hematology, serum biochemistry, organs weights, macroscopic and microscopic examinations were examined.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	>= 50 mg/kg bw/day (nominal)	test mat.	male/female	Black feces revealed in males and females in 0.5 mg/kg or more groups, and Grayish green/Dark green contents in cecum, colon and rectum at autopsy in males and females in 5 mg/kg or more groups. However, there were no systemic toxic signs together with those changes in males and females in all groups.

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

no effects

Food efficiency

not examined

Water consumption and compound intake (if drinking water study)

not examined

Ophthalmoscopic examination

not examined

Haematology

no effects

Clinical chemistry

no effects

Urinalysis

no effects

Neurobehaviour

no effects

Organ weights

no effects

Gross pathology

no effects

Histopathology: non-neoplastic

no effects

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY:

[Observation of administration period]: No death was observed in both sexes. Black feces were observed in both sexes given 0.5 mg/kg bw/day or more groups.

[Observation of recovery period]: No death was observed in both sexes. No abnormal clinical signs were observed in both sexes.

BODY WEIGHT AND WEIGHT GAIN: (See table 1)

No effect was observed.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): (See table 1)

No effect was observed.

HAEMATOLOGY : (See table 3) [Examinations at the end of administration period]

- Male: No effect was observed.
 - Female: The eosinophil counts were significantly low in the 5 and 50 mg/kg groups. The decrease in the eosinophil counts was not clinically or toxicologically important, and histopathological examination revealed no findings indicative of a change in hematopoiesis. Thus, the significant difference was considered to be incidental.
- [Examination at the end of recovery period]
- Male: The platelet counts were significantly high in the 50 mg/kg group.
 - Female: The high Ht values and Low monocyte counts were observed in the 50 mg/kg group.

CLINICAL CHEMISTRY : (See table 4)[Examinations at the end of administration period]

- Male: The γ -GTP was significantly low in the 0.5 mg/kg groups, which was not dose-dependent.
 - Female: The γ -globulin fraction was significantly low in the 50 mg/kg group. This was not accompanied by significant differences in other fractions, and no changes were noted in white blood cell counts, etc. Therefore, the significant difference was considered to be incidental.
- [Examination at the end of recovery period]
- Male: The ALT, Na and Cl were significantly low in the 50 mg/kg group.
 - Female: The TP, albumin and Ca were significantly low in the 50 mg/kg group.

URINALYSIS: No effect was observed.

NEUROBEHAVIOUR:

[Observation of administration period]: No effect was observed.

[Observation of recovery period]:

- Male: No effect was observed.
- Female: The spontaneous locomotor activity was significantly low in the 50 mg/kg group at the time point of 50-60 minutes after the start of measurement.

ORGAN WEIGHTS:

[Examinations at the end of administration period]:

No effect was observed.

[Examination at the end of recovery period]

- Male: No effect was observed.
- Female: The liver absolute and relative weights were significantly high in the 50 mg/kg group.

GROSS PATHOLOGY :

[Examinations at the end of administration period]:

- Male: Grayish green/Dark green contents in cecum, colon and rectum at autopsy in 5 mg/kg or more groups. The swelling of the submandibular lymph node was observed in 1 male in the 0.5 mg/kg group. This change was not dose-dependent and was considered not toxicologically important.
- Female: Grayish green/Dark green contents in cecum, colon and rectum at autopsy in 5 mg/kg or more groups.

[Examination at the end of recovery period]

- Male: No effect was observed.
- Female: The black patches in the glandular stomach were observed in 1 female in the 50 mg/kg group.

HISTOPATHOLOGY: (See table 2) [Examinations at the end of administration period]:

The slight microgranuloma in the liver, etc., were observed in males and females in the control and 50 mg/kg groups. The incidences and grades of these changes were within the normal ranges. [Examination at the end of recovery period] The slight microgranuloma in the liver, etc., were observed in males and females in the control and 50 mg/kg groups. The incidences and grades of these changes were within the normal ranges. At the end of the recovery period, significant differences which were not noted at the end of dosing period were noted in the spontaneous locomotor activity, haematology, clinical chemistry, or organ

weights. These were not correlated and not accompanied by histopathological findings. Therefore, these significant differences were considered not toxicologically important. One female in the 50 mg/kg group showed black patches in the glandular stomach, which was not noted at the end of dosing and was considered not related to the test substance administration. In females in the 50 mg/kg group, absolute and relative liver weights were significantly low, which were not noted at the end of dosing and the significant differences were considered incidental.

Any other information on results incl. tables

Table 1 Body weight and food consumption in rats dosed with MWCNT by gavage for 28 days

Dose (mg/kg bw/day)	Male				Female			
	0	0.5	5	50	0	0.5	5	50
Body weight(g)								
Day 1	183.7 ± 11.1	185.7 ± 9.7	186.7 ± 11.4	186.8 ± 8.4	149.7 ± 8.6	150.2 ± 10.8	150.5 ± 10.3	149.0 ± 10.3
Day 4	212.9 ± 13.3	213.2 ± 10.9	215.5 ± 14.0	215.0 ± 10.9	161.7 ± 9.9	161.8 ± 9.4	160.8 ± 10.7	159.1 ± 10.8
Day 7	242.0 ± 15.0	242.0 ± 14.4	244.5 ± 13.8	241.5 ± 13.0	172.0 ± 11.9	172.7 ± 15.4	172.0 ± 15.9	168.7 ± 13.3
Day 14	305.7 ± 18.5	304.5 ± 16.8	308.3 ± 17.5	305.3 ± 17.3	197.4 ± 12.3	193.0 ± 19.8	198.0 ± 19.9	194.0 ± 17.7
Day 21	362.4 ± 20.0	359.5 ± 17.2	367.2 ± 23.3	359.7 ± 24.1	219.7 ± 16.1	215.2 ± 22.1	218.8 ± 22.0	213.2 ± 19.9
Day 28	399.7 ± 24.1	392.2 ± 21.8	401.8 ± 30.0	394.5 ± 27.2	237.8 ± 18.7	227.7 ± 17.3	236.8 ± 23.2	230.4 ± 22.2
Food consumption (g/rat/day)								
Day 0 - 1	22.42 ± 2.02	22.00 ± 2.37	22.00 ± 1.67	22.58 ± 1.56	16.42 ± 2.19	16.83 ± 2.64	17.00 ± 1.26	17.50 ± 2.39
Day 1 - 4	23.33 ± 1.49	23.33 ± 1.45	23.90 ± 1.62	23.33 ± 1.12	16.76 ± 1.17	16.38 ± 0.98	16.17 ± 1.60	16.48 ± 1.68
Day 4 - 7	25.36 ± 1.75	24.95 ± 2.02	24.98 ± 1.55	24.65 ± 1.17	16.96 ± 1.39	16.77 ± 1.85	17.00 ± 2.31	17.10 ± 1.86
Day 7 - 14	26.94 ± 1.92	26.20 ± 1.62	26.53 ± 1.99	26.14 ± 1.18	18.27 ± 1.47	17.22 ± 1.02	17.92 ± 2.22	17.74 ± 1.82
Day 14 - 21	28.98 ± 1.42	28.25 ± 1.47	28.33 ± 3.34	27.69 ± 1.44	19.31 ± 1.96	18.50 ± 1.52	18.83 ± 2.33	18.62 ± 2.30
Day 21 - 28	29.09 ± 1.89	27.62 ± 2.14	28.18 ± 2.94	28.03 ± 2.26	19.66 ± 1.94	18.62 ± 1.86	19.20 ± 2.66	19.32 ± 2.51

Table 2 Histopathological findings of rats dosed with MWCNT by gavage for 28 days

Sex		Male				Female			
		0	0.5	5	50	0	0.5	5	50
Dose (mg/kg bw/day)									
	Grade								
Number of animals examined (a)		6	0	1	6	6	0	0	6
Lung	+								
Aggregation of alveolar macrophage	+	1	-	-	0	0	-	-	0
Mineralization of artery	+	0	-	-	0	1	-	-	0
Pancreas									
Atrophy of focal acinar cell	+	1	-	-	0	0	-	-	0
Ileum									
Diverticulum	+	0	-	1	0	1	-	-	0
Liver									
Microgranuloma	+	3	-	-	2	4	-	-	2
Kidney									
Tubular epithelium regeneration	+	2	-	-	1	0	-	-	0
Hyaline droplet in proximal tubular epithelium	+	0	-	-	1	0	-	-	0

Eosinophilic body in proximal tubular epithelium	+	0	-	-	1	0	-	-	0
Focal inflammatory cell infiltration in cortex	+	0	-	-	0	0	-	-	1

Grade: + slight change, - Not applicable

a): The histopathological examination was carried out in 6 animals at 0 and 50 mg/kg bw/day and in animal which showed gross findings at 0.5 and 5 mg/kg bw/day.

Table 3 Hematology in rats dosed with MWCNT by gavage for 28 days

Dose (mg/kg bw/day)	Male				Female			
	0	0.5	5	50	0	0.5	5	50
RBC (x10 ⁴ /ml)	878.8 ± 39.3	865.2 ± 27.7	862.8 ± 18.9	878.47 ± 35.5	879.43 ± 30.3	866.8 ± 48.2	855.8 ± 15.6	864.5 ± 24.0
Hemoglobin (g/dl)	17.20 ± 0.45	16.97 ± 0.56	17.12 ± 0.433	17.08 ± 0.28	16.77 ± 0.45	16.90 ± 0.82	16.50 ± 0.24	16.58 ± 0.43
Hematocrit (%)	48.57 ± 0.88	48.00 ± 1.56	48.72 ± 0.81	47.95 ± 1.09	46.13 ± 0.94	46.77 ± 1.94	45.98 ± 0.79	45.80 ± 1.45
MCV (fl)	55.33 ± 1.73	55.52 ± 1.89	56.47 ± 0.87	54.62 ± 1.86	52.48 ± 1.39	54.05 ± 2.16	53.73 ± 0.92	52.98 ± 1.12
MCH (pg)	19.58 ± 0.47	19.60 ± 0.41	19.85 ± 0.18	19.45 ± 0.68	19.07 ± 0.54	19.50 ± 0.68	19.30 ± 0.39	19.20 ± 0.37
MCHC (g/dl)	35.42 ± 0.40	35.35 ± 0.58	35.13 ± 0.30	35.63 ± 0.24	36.35 ± 0.38	36.12 ± 0.33	35.88 ± 0.40	36.22 ± 0.37
Reticulocyte (%)	3.683 ± 0.405	3.603 ± 0.448	3.545 ± 0.534	3.445 ± 0.434	2.907 ± 0.730	3.265 ± 0.431	3.013 ± 0.557	2.875 ± 0.644
WBC (10 ² /μl)	128.57 ± 38.56	116.95 ± 15.35	116.48 ± 29.80	129.37 ± 27.96	91.07 ± 12.67	109.07 ± 27.58	88.83 ± 26.32	79.32 ± 18.52
Differential leukocyte counts (x 10 ² /μl)								
Lymphocyte	111.07 ± 35.91	100.13 ± 12.75	102.98 ± 27.80	111.30 ± 23.02	78.73 ± 11.18	95.97 ± 27.81	78.45 ± 26.20	68.18 ± 18.27
Neutrophil	12.63 ± 3.89	11.82 ± 2.65	9.95 ± 2.83	13.88 ± 5.17	9.32 ± 3.07	10.58 ± 4.12	8.48 ± 2.78	8.88 ± 4.68
Eosinophil	1.43 ± 1.02	1.67 ± 0.31	1.18 ± 0.64	1.07 ± 0.39	1.68 ± 0.89	1.08 ± 0.41	0.87 ± 0.33*	0.88 ± 0.34*
Basophil	0.07 ± 0.05	0.00 ± 0.00	0.03 ± 0.05	0.02 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.04	0.00 ± 0.00
Monocyte	3.37 ± 0.81	3.33 ± 0.76	2.33 ± 1.00	3.10 ± 0.88	1.33 ± 0.62	1.43 ± 0.48	1.02 ± 0.52	1.37 ± 1.18

RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin concentration, WBC: White blood cell

*: significantly different from control group (P<0.05)

Table 4 Serum biochemistry in rats doses with MWCNT by gavage 28 days.

Dose (mg/kg bw/day)	Male				Female			
	0	0.5	5	50	0	0.5	5	50
T. protein (g/dl)	5.67 ± 0.18	5.65 ± 0.12	5.57 ± 0.23	5.70 ± 0.28	5.80 ± 0.32	5.65 ± 0.23	5.87 ± 0.21	5.75 ± 0.16
Glucose (mg/dl)	164.7 ± 8.2	162.2 ± 18.9	168.3 ± 16.8	173.5 ± 13.9	137.3 ± 17.3	134.5 ± 20.1	136.5 ± 13.4	138.8 ± 18.6
Triglyceride (mg/dl)	50.7 ± 11.5	48.7 ± 18.3	39.7 ± 13.5	44.7 ± 9.5	10.0 ± 2.5	11.2 ± 3.7	10.7 ± 4.1	11.2 ± 5.0
T. cholesterol (mg/dl)	59.5 ± 12.1	51.7 ± 9.3	56.8 ± 11.4	56.0 ± 10.1	56.0 ± 5.8	56.7 ± 9.0	60.8 ± 7.7	62.8 ± 13.7
BUN (mg/dl)	14.42 ± 2.12	13.53 ± 2.22	14.32 ± 1.57	15.22 ± 2.61	16.02 ± 1.36	17.22 ± 2.80	16.08 ± 4.45	15.77 ± 4.60
Creatinine (mg/dl)	0.497 ± 0.027	0.483 ± 0.023	0.502 ± 0.035	0.502 ± 0.022	0.505 ± 0.037	0.513 ± 0.043	0.507 ± 0.058	0.508 ± 0.045
T. bilirubin (mg/dl)	0.047 ±	0.038 ±	0.043 ±	0.040 ±	0.055 ±	0.063	0.060 ±	0.057 ±

	0.008	0.008	0.008	0.009	0.012	± 0.012	0.021	0.008
AST (IU/l)	67.3 ± 7.4	64.2 ± 7.7	66.3 ± 4.2	67.7 ± 10.0	69.3 ± 5.2	65.0 ± 4.7	61.5 ± 5.8	69.7 ± 11.6
ALT (IU/l)	26.2 ± 3.2	26.2 ± 2.6	25.7 ± 2.6	25.2± 2.6	20.3 ± 2.9	22.7 ± 2.4	21.2 ± 3.4	22.2± 4.4
ALP (IU/l)	688.8 ± 46.3	693.3 ± 122.5	614.2 ± 112.8	700.7 ± 171.2	423.8 ± 44.8	412.8 ± 91.3	333.2 ± 65.3	398.7± 138.4
γ-GTP (IU/l)	0.85 ± 0.21	0.62 ± 0.12*	0.68 ± 0.12	0.70 ± 0.14	1.22 ± 0.19	1.18 ± 0.12	0.95 ± 0.20	1.20± 0.27
Calcium (mg/dl)	10.18 ± 0.30	9.90 ± 0.14	9.93 ± 0.21	10.07 ± 0.27	9.88 ± 0.08	9.87 ± 0.46	10.00 ± 0.39	9.78 ± 0.43
I.phosphorus (mg/dl)	8.78 ± 0.49	8.67 ± 0.22	8.53 ± 0.48	8.77 ± 0.36	8.03 ± 0.42	8.02 ± 0.67	7.93 ± 0.38	7.70± 0.91
Sodium (mEq/l)	143.2 ± 1.2	143.2 ± 0.8	143.3 ± 0.8	143.8 ± 1.0	144.0 ± 1.4	143.5 ± 1.9	143.8 ± 1.5	144.3 ± 0.8
Potassium (mEq/l)	4.873 ± 0.268	4.923 ± 0.265	4.858 ± 0.117	4.762 ± 0.331	4.593 ± 0.372	5.108 ± 0.420	4.862 ± 0.351	4.755± 0.500
Chloride (mEq/l)	104.3 ± 1.9	105.0 ± 0.6	105.2 ± 1.2	105.0 ± 0.9	107.3 ± 2.1	107.2 ± 1.9	106.8 ± 1.9	106.7 ± 1.6
Albumin (%)	51.03 ± 2.11	51.93 ± 1.67	52.93 ± 1.25	53.50 ± 2.68	54.83 ± 2.31	55.53 ± 1.87	55.65 ± 1.94	55.88 ± 1.40
Alpha-1 globlin (%)	22.93 ± 3.10	23.35 ± 1.51	21.20 ± 2.81	21.47 ± 3.08	17.60 ± 2.54	17.93 ± 2.17	19.28 ± 2.38	19.32± 0.65
Alpha-2 globlin (%)	7.03 ± 0.34	6.65 ± 0.45	7.10 ± 0.51	6.98 ± 0.55	6.95 ± 0.77	7.45 ± 0.29	6.40 ± 0.75	6.57 ± 0.45
Beta globlin (%)	15.48 ± 0.98	14.72 ± 1.09	15.15 ± 1.1	14.48 ± 0.44	15.27 ± 0.85	14.57 ± 0.99	14.40 ± 1.11	14.35± 0.9
Gamma globlin (%)	3.52 ± 0.60	3.35 ± 0.63	3.62 ± 0.95	3.57 ± 0.43	5.35 ± 1.29	4.52 ± 0.76	4.27± 1.04	3.88 ± 0.67*
Albumin/Globlin	1.045 ± 0.089	1.083 ± 0.074	1.123 ± 0.057	1.153 ± 0.128	1.218 ± 0.115	1.252 ± 0.091	1.257 ± 0.101	1.268 ± 0.070

BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alaine aminotransferase

AIP: Alkaline phosphatase

*: significantly different from control group (P<0.05)

Overall remarks, attachments

Attached full study report

Attached full study report
CNT oral dose.pdf / 900.97 KB (application/pdf): Appendix 4

Applicant's summary and conclusion

Conclusions

Rats were dosed with MWCNT once daily by gavage for 28 days with a 14-day recovery period, and no toxicological effects were found up to the highest dose tested. However, the possibility that CNTs dosed by oral administration are absorbed from the gastro-intestinal tract and persistent in the body for the long term cannot be ruled out, and a further study for chronic oral toxicity will be helpful to confirm the safety of CNTs.

Endpoint study record: Nikkiso MWCNT: Repeated dose toxicity: inhalation.001**Administrative Data**

Purpose flag key study

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies According to OECD TG412

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST		NEDO Project Report		University of Occupational and Environmental Health, Japan				
publication	Morimoto Y, Hirohashi M, Ogami A, Oyabu T, Myojo T, Todoroki M, Yamamoto M, Hashiba M, Mizuguchi Y, Lee BW, Kuroda E, Shimada M, Wang WN, Yamamoto K, Fujita K, Endoh S, Uchida K, Kobayashi N, Mizuno K, Inada M, Tao H, Nakazato T, Nakanishi J, Tanaka I.	2012	Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation	Nanotoxicology, 6(6):587-599, (2012)					
publication	Oyabu T, Myojo T, Morimoto Y, Ogami A, Hirohashi M, Yamamoto M, Todoroki M, Mizuguchi Y, Hashiba M, Lee BW, Shimada M, Wang WN, Uchida K, Endoh S, Kobayashi N, Yamamoto K, Fujita K, Mizuno K, Inada M, Nakazato T, Nakanishi J, Tanaka I.	2011	Biopersistence of inhaled MWCNT in rat lungs in a 4-week well-characterized exposure	Inhalation Toxicology, 23 (13) 784-791, (2011)					

Data access

data published

Materials and methods**Test type**

subchronic

Limit test

no

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:	Nikkiso MWCNT

Test material form

dispersion

Details on test material

- Name of test material (as cited in study report): MWCNT
- Impurities (identity and concentrations): Ca: 176 ppm, Al: 80 ppm, Fe: 53 ppm, Cd: 16 ppm, Li: 0.5 ppm
- Other: The MWCNTs were synthesized by catalytic chemical vapor deposition (CVD) method.[Bulk MWCNTs]:Diameter: 44 nm, BET surface area: 69 ± 37 m²/g, D/G ratio: 0.078[Dispersed MWCNTs in solution]:Diameter: 48 nm, Length: 0.94 μ m, D/G ratio: 0.174[MWCNTs aerosol in exposure chamber]:Diameter: 63 nm, Length: 1.1 μ m, Mass concentration: 0.37 ± 0.18 mg/m³

Test animals**Species**

rat

Strain

Wistar

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Kyudo Co., Ltd., Japan
- Age at study initiation: 9 weeks
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 5days

Administration / exposure

Route of administration

inhalation: aerosol

Type of inhalation exposure

whole body

Vehicle

clean air

Duration of treatment / exposure

4 weeks

Frequency of treatment

6hrs/d x 5d/w

Doses/concentrations

0.37 ± 0.18 mg/m³

Basis analytical conc.

MMAD / GSD

Diameter: 63 nm, Length: 1.1 µm, Mass concentration:
0.37 ± 0.18 mg/m³

No. of animals per sex per dose

15 male rats per observation period

Control animals

yes, concurrent no treatment

yes, concurrent vehicle

other: Nickel oxide

Details on study design

- Post-exposure recovery period in satellite groups: 3, 28, and 91 days

Positive control

Nickel oxide

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: No data

DETAILED CLINICAL OBSERVATIONS: No data

BODY WEIGHT: No data

FOOD CONSUMPTION: No data

FOOD EFFICIENCY: No data

WATER CONSUMPTION: No data

OPHTHALMOSCOPIC EXAMINATION: No data

HAEMATOLOGY: No data

CLINICAL CHEMISTRY: No data

URINALYSIS: No data

NEUROBEHAVIOURAL EXAMINATION: No data

Sacrifice and pathology

GROSS PATHOLOGY: No data

ORGAN WEIGHT: Lung, liver, kidney, spleen, testis, brain

HISTOPATHOLOGY: Yes

- Lung, liver, kidney, spleen, testis, brain

Other examinations

MWCNT amount: The left lung of 5 rats from each group was used for the determination of MWCNT amount. Chemokine of lung and BALF: A part of the right lung was used for inflammatory chemokine release. The other 5 rats provided bronchoalveolar lavage.

Statistics

Statistical analysis was carried out using the Mann-Whitney test with differences of $p < 0.05$ considered to be statistically significant.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEC	0.37 mg/m ³ air	test mat.	male	For the MWCNTS exposure group (0.37mg/m ³), the lungs weight and neutrophil in BALF and lung tissue showed a transient change only at the third day after the exposure, but no significant change was observed about other observation items.

Results of examinations

Clinical signs and mortality

no data

Body weight and weight gain

no data

Food consumption

not examined

Food efficiency

not examined

Water consumption

not examined

Ophthalmoscopic examination

not examined

Haematology

no data

Clinical chemistry

no data

Urinalysis

no data

Neurobehaviour

no data

Organ weights

yes

Gross pathology

no data

Histopathology: non-neoplastic

no effects

Histopathology: neoplastic

not examined

Details on results**ORGAN WEIGHTS:**

At 3 days after 4-week inhalation, both the wet and dry lung weight of the MWCNT exposure group increased compared with those of clean air group, but at 1 and 3 months after inhalation both weights were returned to control level and no static change was observed.

HISTOPATHOLOGY:

In an MWCNT exposure group, neutrophil infiltration into alveolar space, granulomatous lesion, or interstitial collagen deposition was not found during the observation period. However, although only to a slight extent, alveolar macrophages that ingested MWCNT were observed. Even in the Triton group, the result was practically the same as for the unexposed group and nothing unusual was observed throughout the period.

MORPHOLOGICAL FEATURE OF ALVEOLAR MACROPHAGE BY TEM:

In an alveolar macrophage, MWCNT was found in phagolysosomes. Some of the MWCNT was agglomerated, but some was individual. MWCNT was not observed in nuclei or organelles.

BALF:

There was no significant difference in the total cells among the unexposed group, the Triton group and the MWCNT group. Although the MWCNT group showed the increasing tendency at 3 days, there was also no significant difference in the neutrophils counts among the three groups. Significant increase in ALP release in BALF in the MWCNT group was not observed through the observation period. The concentration of CINC-1 in lung tissue in the MWCNT group was significantly increased over the unexposed group at 3 days. The Triton group showed significant decreases at 3 days and 1 month, reaching the same level as the unexposed group 3 months later. Regarding the CINC-2 concentration, the MWCNT group showed a significant increase over the unexposed group at 3 days. As for the CINC-3 concentration in lung tissue, the MWCNT group also showed a significant and transient increase over the unexposed group at 3 days. As for the MPO concentration, the MWCNT group also showed a significant increase over the unexposed group at 3 days.

Overall remarks, attachments**Attached full study report****Illustration (picture/graph)**

Applicant's summary and conclusion

Conclusions

In the inhalation study, rats were exposed to 0.37 mg/m³ aerosols of well-dispersed MWCNTs (>70% of MWCNTs were individual fibers) for 4 weeks, and were sacrificed at 3 days, 1 month, and 3 months after the end of exposure. For the MWCNTS exposure group (0.37mg/m³), the lungs weight and neutrophil in BALF and lung tissue showed a transient change only at the third day after the exposure, but no significant change was observed about other observation items. The inhalation exposures delivered less amounts of MWCNTs into the lungs, and therefore less pulmonary inflammation responses was observed, as compared to intratracheal instillation.

Endpoint study record: Hanwha CM-100: Repeated dose toxicity: inhalation.001

Administrative Data

supporting study

Purpose flag

Study result type

experimental result **Study period** 2011

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	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 (2): 439-448, (2012)					

Data access

data published

Materials and methods

GLP compliance

no data

Test materials

Test material identity

Identifier	Identity
other:	Hanwha CM-100

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

Type of inhalation exposure

whole body

Vehicle

other: water

Duration of treatment / exposure

5 days

Frequency of treatment

6 hr/day, 5 day/week

Doses/concentrations

0, 0.16, 0.34, 0.94 mg/m³

Basis analytical conc.

No. of animals per sex per dose

10 rats

Details on study design

- Post-exposure recovery period in satellite groups: 1 month

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: No data

DETAILED CLINICAL OBSERVATIONS: No

BODY WEIGHT: Yes

- Time schedule for examinations: One day prior to exposure, once a week during the inhalation exposure and recovery, and before necropsy. animal autopsies were conducted on day 0 and 1 month after the 5-day exposure.

FOOD CONSUMPTION: No data

WATER CONSUMPTION: No data

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

CLINICAL CHEMISTRY: No
URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No

OTHER:

Analysis of H₂O₂ Concentration in Bronchoalveolar Lavage.
Enhanced-darkfield light microscopy imaging of MWCNTs.
Lung Cell Isolation.
Single-Cell Gel Electrophoresis (Comet Assay).

Sacrifice and pathology

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes

Statistics

The statistical analyses were performed using SPSS 12.1, and the data expressed as the mean $\pm$ standard error (SE). A one-way analysis of variance was applied to test all the data. A value of $p < 0.05$ indicated statistical significance.

Results and discussions

Results of examinations

Clinical signs and mortality

no data

Body weight and weight gain

no effects

Food consumption

not examined

Food efficiency

not examined

Water consumption

not examined

Ophthalmoscopic examination

not examined

Haematology

not examined

Clinical chemistry

not examined

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

no data

Gross pathology

no data

Histopathology: non-neoplastic

no data

Histopathology: neoplastic

not examined

Details on results

The animals exhibited no significant body weight changes, abnormal clinical signs, or mortality during the experiment.

Measurement of H₂O₂ Concentration in BAL:

Although the H₂O₂ concentrations did not show any statistical significance with any of the MWCNT concentrations when compared with the negative fresh-air control, there were some increasing trends.

At one month after the 5-day exposure, the H₂O₂ concentration exhibited an increasing trend, although there was no statistical significance. Furthermore, the H₂O₂ concentrations increased after 1 month postexposure to the low and high MWCNT concentrations when compared with those following the 5-day exposure, plus a statistical significance was found in the H₂O₂ concentrations between 1 month postexposure and following the 5-day exposure. Potential lung deposition of MWCNTs: The MWCNT-exposed lungs showed that the MWCNTs were deposited in the alveolar epithelium and the alveolar macrophages after the 5-day inhalation exposure (arrows). The deposition of the MWCNTs also persisted even after 30 days postexposure, although the deposition amount was significantly reduced.