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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 1 -**

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

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ENV/JM/MONO(2015)12/PART1
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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 1 -**

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

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

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

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

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

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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World Wide Web site (www.oecd.org/chemicalsafety/)**

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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; vi) mammalian toxicology; and vii) material safety. These endpoints were judged to be most important based largely on the general experience of testing chemicals, while taking into account the potentially different or new properties of nanomaterials. It is worth noticing that it was not expected that testing for all of the listed endpoints would be necessary for each of the selected MNs.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FOREWORD

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

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

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

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

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

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

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

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

ACKNOWLEDGMENTS

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

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PART 2

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PART 3

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APPENDIX	ENV/JM/MONO(2015)12/PART3

1. GENERAL INFORMATION

1.1 Identification

Substance 1 identification: MWCNT_Baytubes

Chemical name MWCNT_Baytubes

Public name Multiwalled Carbon Nanotubes

Type of substance 1

Composition mono constituent substance

Origin inorganic

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	Baytubes (Alternate2)		

Substance 2 identification: MWCNT_Graphistrength C100

Chemical name MWCNT_Graphistrength C100

Public name Multiwalled Carbon Nanotubes

Type of substance 2

Composition mono constituent substance

Origin inorganic

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	Arkema Graphistrength C100 (Principal2)		

Substance 3 identification: MWCNT_Mitsui MWNT-7

Chemical name MWCNT_Mitsui MWNT-7

Public name Multiwalled Carbon Nanotubes

Type of substance 3

Composition mono constituent substance

Origin inorganic

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	Arkema Graphistrength C100 (Principal2)		
	Trade name	NANOCYL NC7000 (Principal3)		
	Trade name	Mitsui MWNT-7 (Alternate1)		
	Trade name	Nikkiso MWCNT (Principal 1)		
	Trade name	Baytubes (Alternate2)		
	Trade name	Hanwha MWCNT (Alternate3)		

Substance 4 identification: MWCNT_Nanocyl NC7000

Chemical name MWCNT_Nanocyl NC7000

Public name Multiwalled Carbon Nanotubes

Type of substance 4

Composition mono constituent substance

Origin inorganic

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	NANOCYL NC7000 (Principal3)		

Substance 5 identification: MWCNT_Nikkiso

Chemical name MWCNT_Nikkiso

Public name Multiwalled Carbon Nanotubes

Type of substance 5

Composition mono constituent substance

Origin inorganic

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	Nikkiso MWCNT (Principal 1)		

Substance 6 identification: MWCNT_Hanwha CM-100

Chemical name MWCNT_Hanwha CM-100

Public name Multiwalled Carbon Nanotubes

Type of substance 6

Composition mono constituent substance

Origin inorganic

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	Arkema Graphistrength C100 (Principal2)		
	Trade name	NANOCYL NC7000 (Principal3)		
	Trade name	Mitsui MWNT-7 (Alternate1)		
	Trade name	Nikkiso MWCNT (Principal 1)		
	Trade name	Baytubes (Alternate2)		
	Trade name	Hanwha MWCNT (Alternate3)		

1.2 Composition

Substance 1 composition

Name	Baytubes
Degree of purity	> 95 % (w/w)
Impurities	
Typical concentration < 5 % (w/w)	

Name	
Degree of purity	> 95 % (w/w)

Substance 2 composition

Name	Arkema Graphistrength
Degree of purity	> 92 % (w/w)
Impurities	
Typical concentration < 5 % (w/w)	

Substance 4 composition

Name	Nanocyl NC7000
Degree of purity	> 90 % (w/w)

Substance 5 composition

Name	Nikkiso MWCNT
Degree of purity	> 98 % (w/w)
Impurities	
Reference substance <u>Li</u>	
Typical concentration ca. 0.5 mg/kg	
Reference substance <u>Al</u>	

Typical concentration ca. 80 mg/kg
Reference substance <u>Ca</u>
Typical concentration ca. 176 mg/kg
Reference substance <u>Fe</u>
Typical concentration ca. 53 mg/kg
Reference substance <u>Cd</u>
Typical concentration ca. 16 mg/kg

1.3 Identifiers

1.4 Analytical information

1.5 Joint submission

Joint submission

Lead

US/Japan

1.6 Sponsors

1.7 Suppliers

1.8 Recipients

1.9 Product and process orientated research and development

2. CLASSIFICATION & LABELLING AND PBT ASSESSMENT

2.1 GHS

2.2 DSD - DPD

3. MANUFACTURE, USE AND EXPOSURE

3.1 Technological process

Technological process

Methods of manufacture of substance	of Nikkiso MWCNT: CVD method using floating metal catalyst Arkema Grphistrength of C100: CVD using supported metal catalyst for continuous production by fluidized bedNANOCYLTM NC7000: Catalytic Carbon Vapor Deposition using supported metal catalyst for (semi)-continuous production in a moving bed reactorMitsui-MWNT-7: Floating Chemical Vapor DepositionBaytubesR: Chemical Vapor Deposition using supported metal catalyst for (semi)-continuous production by fluidized bedHanwha MWCNT: Cheaptubes.com MWCNT: Catalytic Carbon Vapor Deposition using supported metal catalyst for (semi)-continuous production in a moving bed reactor
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3.2 Estimated quantities

3.3 Sites

3.4 Information on mixtures

3.5 Life Cycle description

3.6 Uses advised against

3.7 Exposure Scenarios, exposure and risk assessment

3.7.1 Exposure scenarios and local assessment

3.7.2 Environmental assessment for aggregated sources

3.7.3 Generic exposure potential

3.8 Biocidal information

3.9 Emissions Information

3.10 Application for authorisation of uses

4. PHYSICAL AND CHEMICAL PROPERTIES

4.1 Appearance/physical state/colour

Endpoint study record: Bay tubes Appearance/physical state/colour.002

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Actual measurement by BayerMaterials

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from BayerMaterialScience									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: MPMN Guidance Manual	

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
other:	Baytubes

Results and discussion

Physical state at 20°C and 1013 hPa

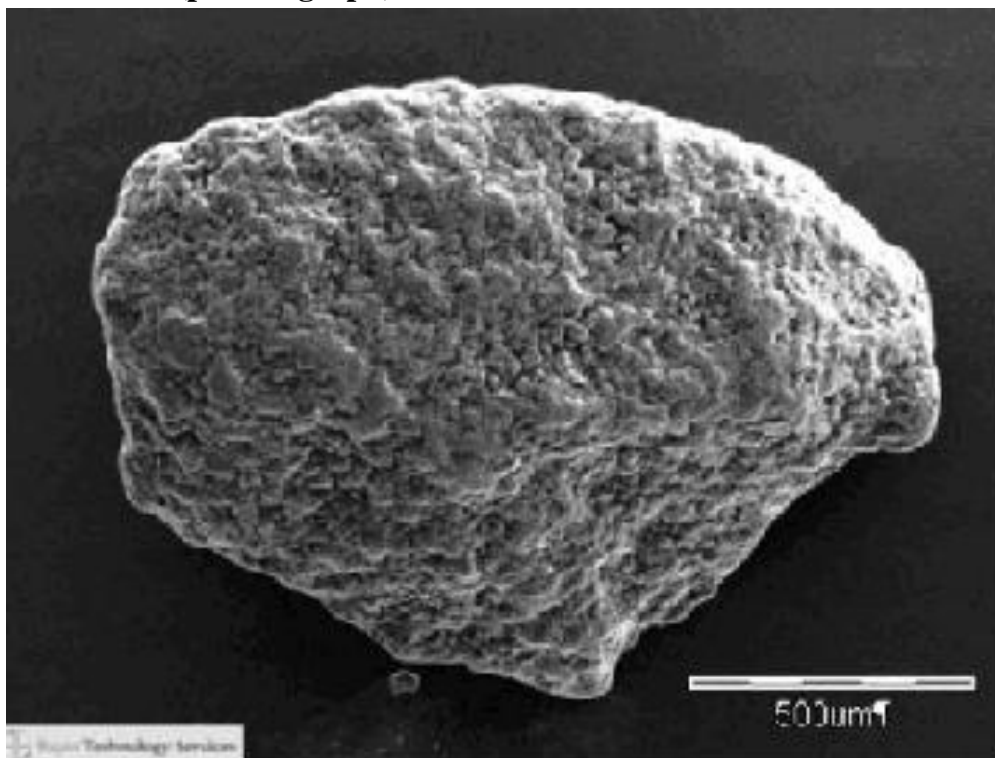
solid

Overall remarks, attachments

Remarks on results including tables and figures

The tube length distribution of MWCNT was measured by Transmission Electron Microscopy after dispersion in aqueous medium after sonication. According to the World Health Organization (WHO) any particle > 5 µm long with an aspect ratio equal to or greater than 3:1 and a diameter < 3 µm is considered a fiber. The mean tube length was 380 - 902 nm (D90 = 980 - 1820 nm). In all cases length D90 was lower than 5 µm.

Illustration (picture/graph)



Endpoint study record: Hodogaya MWNT-7 Appearance/physical state/colour.003

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Experiment results of reliable national institution

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Kobayashi et al.	2010	Biological response and morphological assessment of individually dispersed multi-wall carbon nanotubes in the lung after intratracheal instillation in rats	Toxicology 276 (2010) 143-153					

Materials and methods

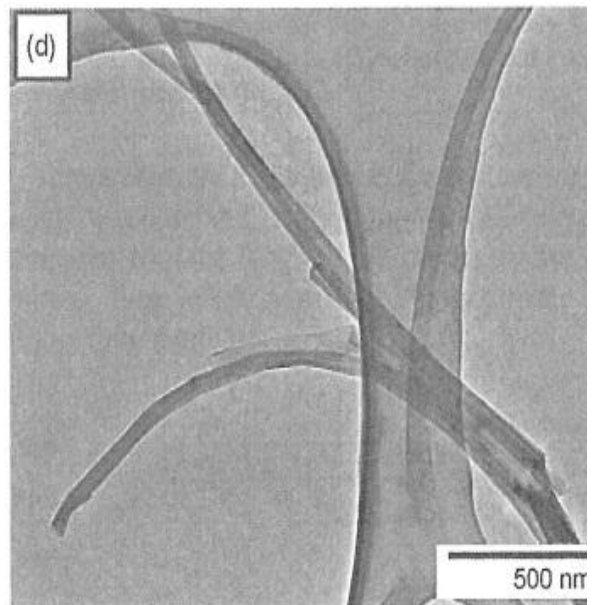
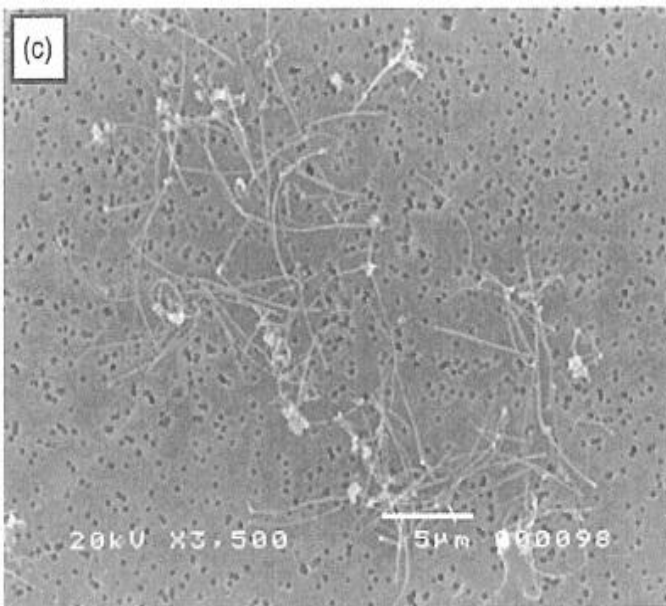
Test materials

Test material identity

Identifier	Identity
other:	Hodogaya (Mitsui) MWNT-7

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: NANOCYL TEM

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Reviewd article

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	L. Ma-Hock, S. Treumann, V. Strauss, S. Brill, F. Luizi, M. Mertler, K. Wiench, A. O. Gamer, B. Ravenzwaay, R. Landsiedel	2009	Inhalation Toxicity of Multiwall Carbon Nanotubes in Rats Exposed for 3 Months	TOXICOLOGICAL SCIENCES 112(2), 468–481 (2009)					

Materials and methods

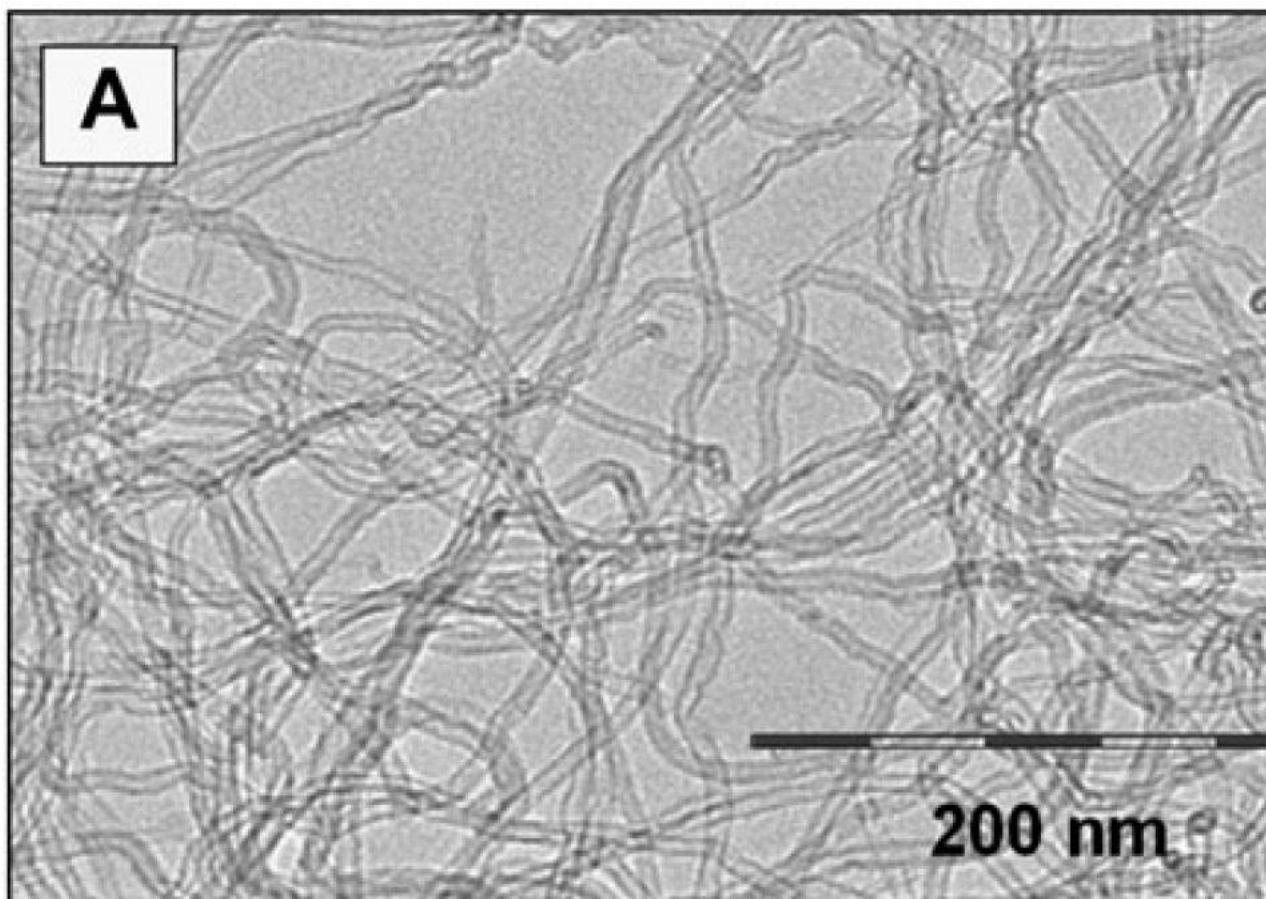
Test materials

Test material identity

Identifier	Identity
	Nanocyl NC7000

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: TEM Picture

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Observation by reliable national institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011	Assessment Report of Nanomaterials -Carbon Nanotubes-	NEDO Project (P06041)					

Materials and methods

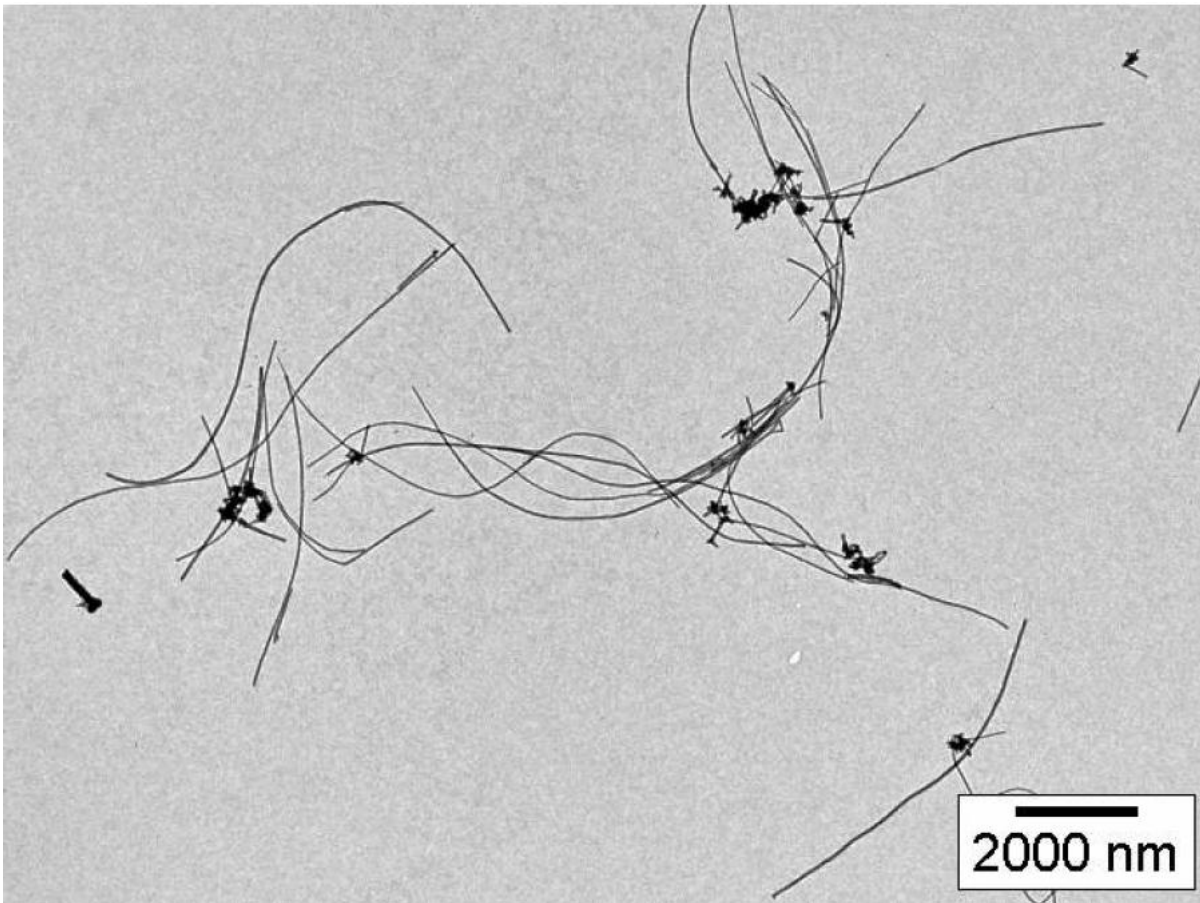
Test materials

Test material identity

Identifier	Identity
	Nikkiso MWCNT

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: Hanwha TEM

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Revised article

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	J. S. Kim, J. H. Sung, K. S. Song, J. H. Lee, S. M. Kim, G. H. Lee, K. H. Ahn, J. S. Lee, J. H. Shin, J. D. Park, I.J. Yu	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	Tox. Sci. 128 (2) 439-448 (2012)					

Materials and methods

Test materials

Test material identity

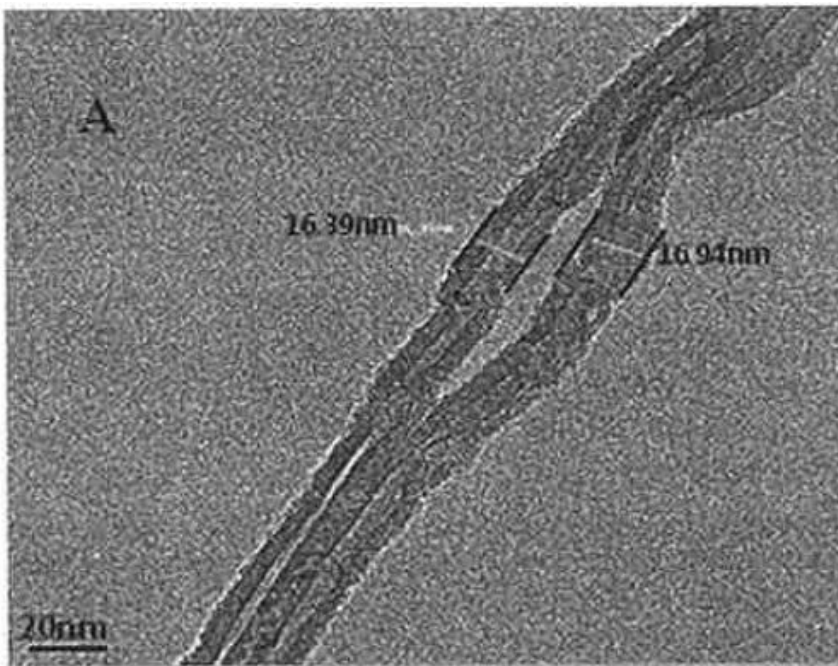
Identifier	Identity
	Hanwha MWCNT

Overall remarks, attachments

Remarks on results including tables and figures

See TEM picture

Illustration (picture/graph)



4.2 Melting point/freezing point

4.3 Boiling point

4.4 Density

4.5 Particle size distribution (Granulometry)

Endpoint study record: Baytubes Particle size distribution (Granulometry).004

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies reviewd article

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	U. Wirtzner, B. Herbold, M. Voetz, J. Ragot	2009	Studies on the in vitro genotoxicity of baytubes, agglomerates of engineered multi-walled carbon-nanotubes (MWCNT)	Tox. Letters 186 (2009) 160-165					

Materials and methods

Type of distribution

volumetric distribution

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Measured by laser diffraction

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

Results and discussions

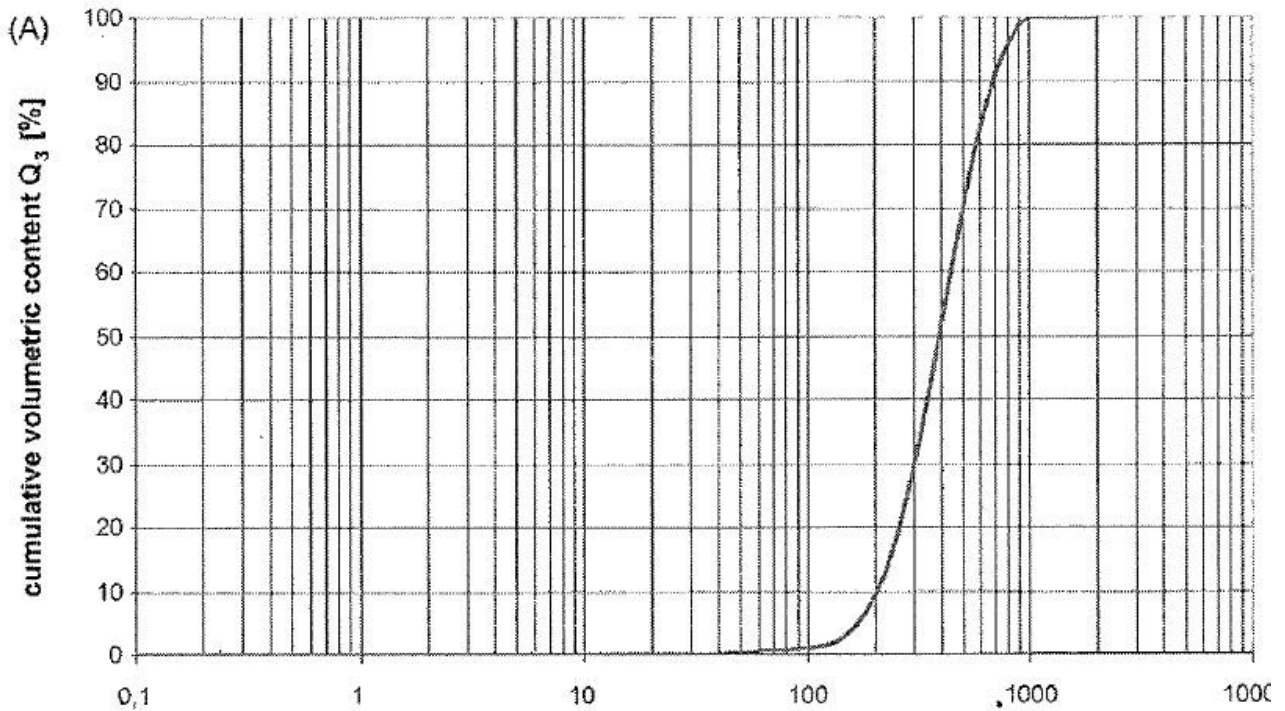
Mass median diameter

ca. 400 μm

Remarks (D50)

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: Graphistrength C100 Particle size distribution (Granulometry).001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	4 (not assignable)
Rationale for reliability incl. deficiencies	Measured by established method

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: internal meathod	

Principles of method if other than guideline

Internal method (NTC DTC 06)

GLP compliance

no data

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Arkema Grapfistrength C100

Results and discussions

Mass median diameter

416.2

µm

Particle size

Percentile	Mean	St. dev.	Remarks
D50	416.2 µm		D(v,0.5)

Endpoint study record: Mitsui NWT-7 Particle size distribution (Granulometry).003

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies Measured by reliable national institute (NHS)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Wako et al.	2010	Effects of preparation methods for multi-wall carbon nanotube (MWCNT) suspensions on MWCNT induced rat pulmonary toxicity	J. Toxicol. Sci. vol. 35, No. 4, pp 437-446					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Measured by SEM

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

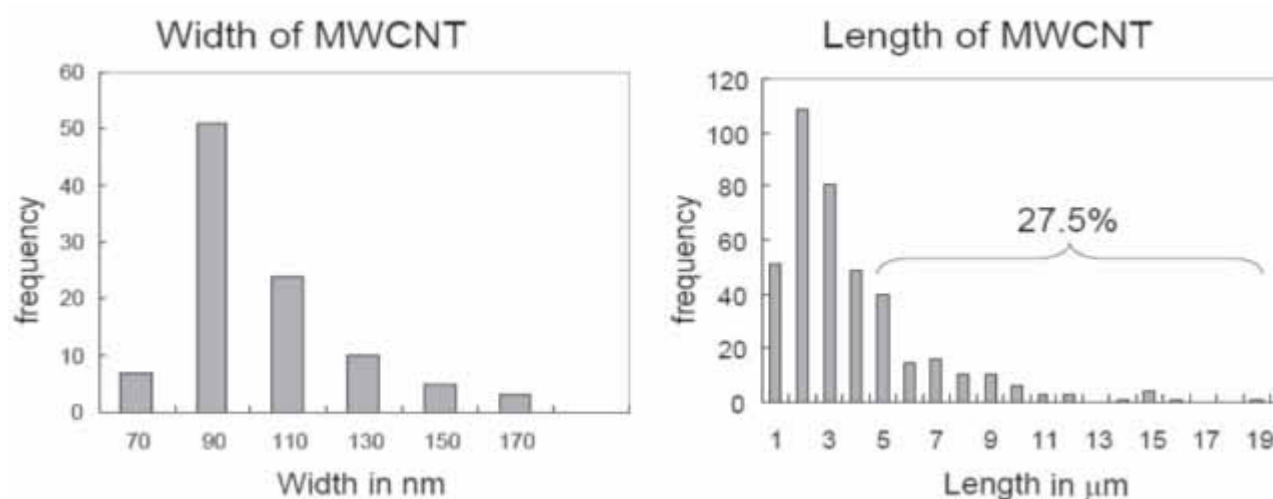
Results and discussions

Mass median diameter

Remarks Diameter 70-170 nm, Length 1-19 um,
>5 um:27.5%

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: nanocyl NC7000 Particle size distribution (Granulometry).002

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Measured by established method

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Report from Nanocyl									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Malvern on dry powder with an injection pressure of 4 bars

GLP compliance

no data

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Nanocyl NC7000

Results and discussions

Mass median diameter

ca. 85 µm

Particle size

Percentile	Mean	St. dev.	Remarks
D10	ca. 31.6 µm		
D50	ca. 85 µm		
D90	ca. 228 µm		

Endpoint study record: Nikkiso Mwcnt Particle size distribution (Granulometry).001

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability deficiencies

incl. The experiment was carried out at the reliable national Institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST	2011	Risk assessment of Nanomaterials -Carbon Nanotubes-						

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Measuterd by TEM

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

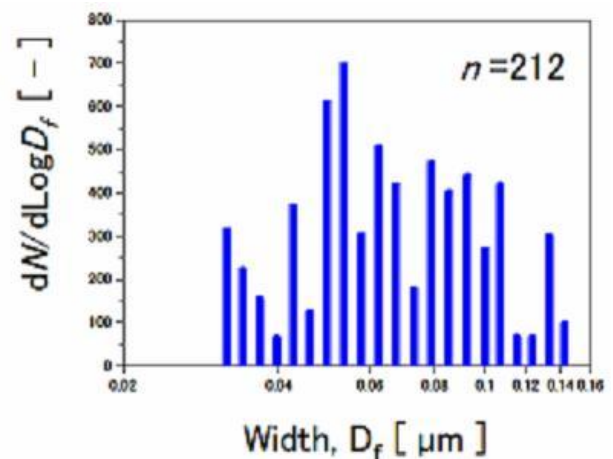
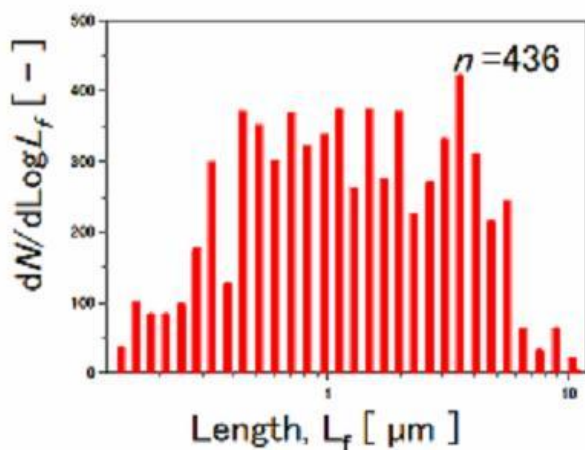
Results and discussions

Any other information on results incl. tables

The size distribution is seen in the attached graph.

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: Hanwha CM100 Particle size distribution (Granulometry).005

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Reviewd article

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	J. S. Kim, J. H. Sung, K. S. Song, J. H. Lee, S. M. Kim, G. H. Lee, K. H. Ahn, J. S. Lee, J. H. Shin, J. D. Park, I.J. Yu	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 Nabotubes (MWCNTs) Generated by New MWCNT Aerosol Generation System	Tox. Sci. 128 (2) 439-448 (2012)					

Materials and methods

Test materials

Test material identity

Identifier	Identity
	Hanwha CM100
	Hanwha MWCNT

Details on test material

Particle size distribution was measured by DLS

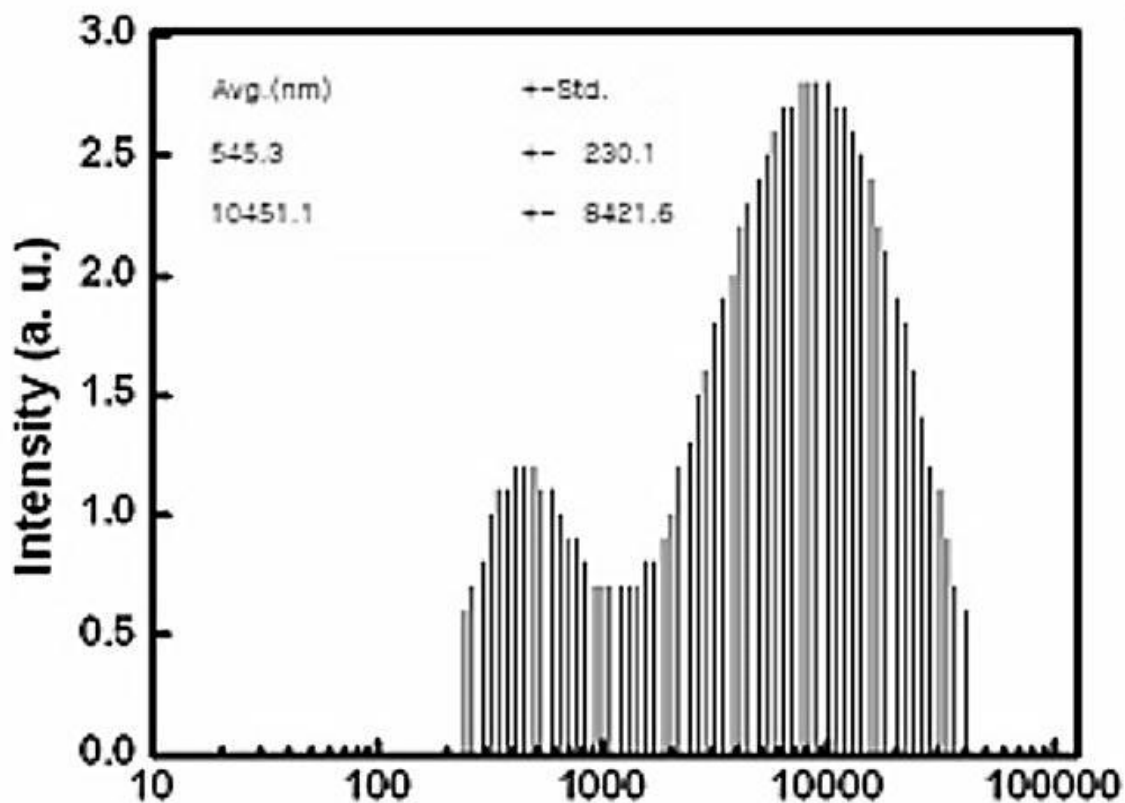
Results and discussions

Any other information on results incl. tables

The main range of length distribution was 543.3 ± 230 and 10451 ± 8421.6 nm, respectively

Overall remarks, attachments

Illustration (picture/graph)



4.6 Vapour pressure

4.7 Partition coefficient

4.8 Water solubility

Endpoint study record: Hanwha CM-100 Water solubility.001

Administrative Data

Purpose flag supporting study

Study result type experimental result

Materials and methods

Test materials

Test material identity

Identifier	Identity
	Hanwha CM100

Details on methods

0.4g of MWCNT was refluxed with 30 mL of nitric acid and 10 mL of hydrogen peroxide in a 100 mL round flask for 10 hrs at 100°C. After refluxing, the MWCNT solution was filtered and diluted with deionised water and stirred for 12 hours. After stirring, MWCNT solution was sonicated for 3 hours in a 20W Bath sonication and stirred for 12 hours. Then, MWCNT solution was diluted with deionised water and filtered.

Results and discussions

Any other information on results incl. tables

Oxidised MWCNT solution was completely dispersed in deionised water.

4.9 Solubility in organic solvents / fat solubility

4.10 Surface tension

4.11 Flash point

4.12 Auto flammability

4.13 Flammability

4.14 Explosiveness

4.15 Oxidising properties

4.16 Oxidation reduction potential

4.17 Stability in organic solvents and identity of relevant degradation products

4.18 Storage stability and reactivity towards container material

4.19 Stability: thermal, sunlight, metals

4.20 pH

4.21 Dissociation constant

4.22 Viscosity

4.23 Additional physico-chemical information

4.24 Agglomeration/aggregation

Endpoint study record: Agglomeration/aggregation.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Tbet et al.	2011	Coating carbon nanotubes with a polystyrene-based polyner protects against pulmonary toxicity	Part Fiber Toxicol. 2011 Jan 21, 8:3.					

Materials and methods

Method

SEM

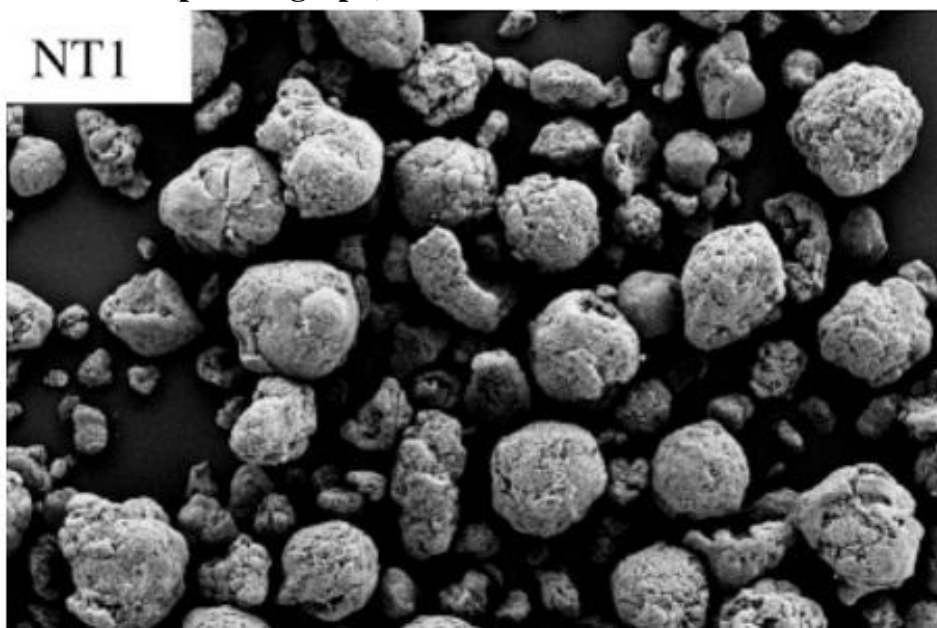
Test materials

Test material Identity

Identifier	Identity
	Arkema Graphistrength C100

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: Agglomeration/aggregation.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Peered paper

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Porter et al	2013	Acute pulmonary dose-responses to inhaled multi-walled carbon nanotubes	Nanotoxicology, Nov. 2013 vol7, pp1179-1104 (Doi; 10.3109/17435390.2012.719649)					

Materials and methods

Principles of method if other than guideline

MOUDI cascade inpacter

Results and discussions

Agglomerate/Aggregate diameter

Mean diameter ca. 1.5 μm

St. Deviation 1.67

Endpoint study record: Agglomeration/aggregation.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Observation by the reliable national institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST	2011	Risk Assessment Report of Nanomaterials - Carbon Nanotube -	NEDO Project (P06041)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: WPMN Guidance Mannual	

Method

SEM

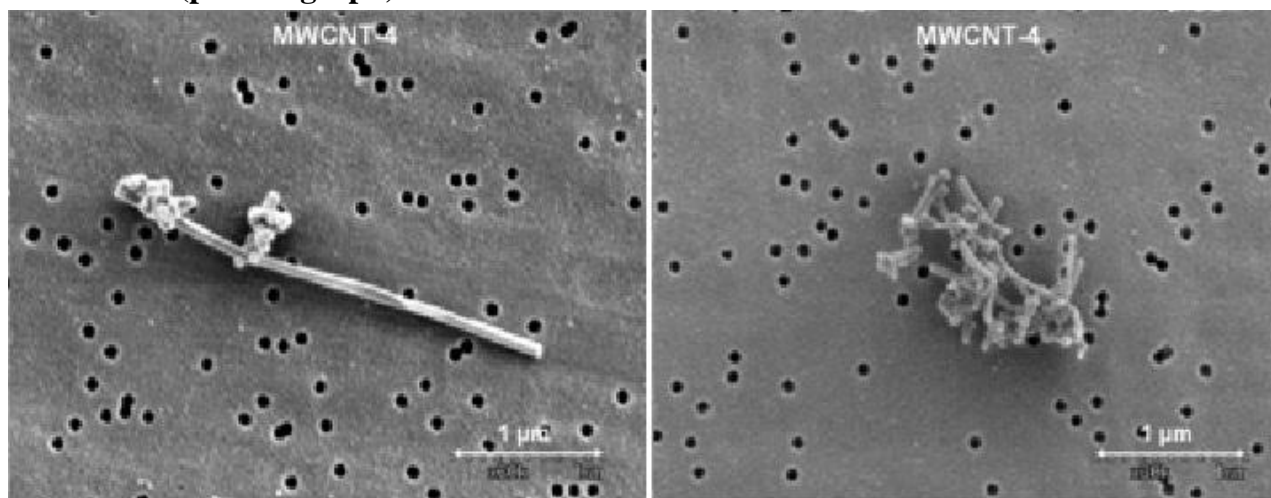
Test materials

Test material Identity

Identifier	Identity
	Nikkiso MWCNT

Overall remarks, attachments

Illustration (picture/graph)



4.25 Crystalline phase

Endpoint study record: Crystalline phase.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Reliable Institute (NIOSH) and Reviewd paper

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Porter et al.	2010	Mouse pulmonary dose- and time course-responses induced by exposure to multi-walled carbon nanotubes	Toxicology 269 (2010) 136–147					

Materials and methods

Method

transmission electron microscopy (TEM)

Test materials

Test material identity

Identifier	Identity
	Mitsui MWNT-7

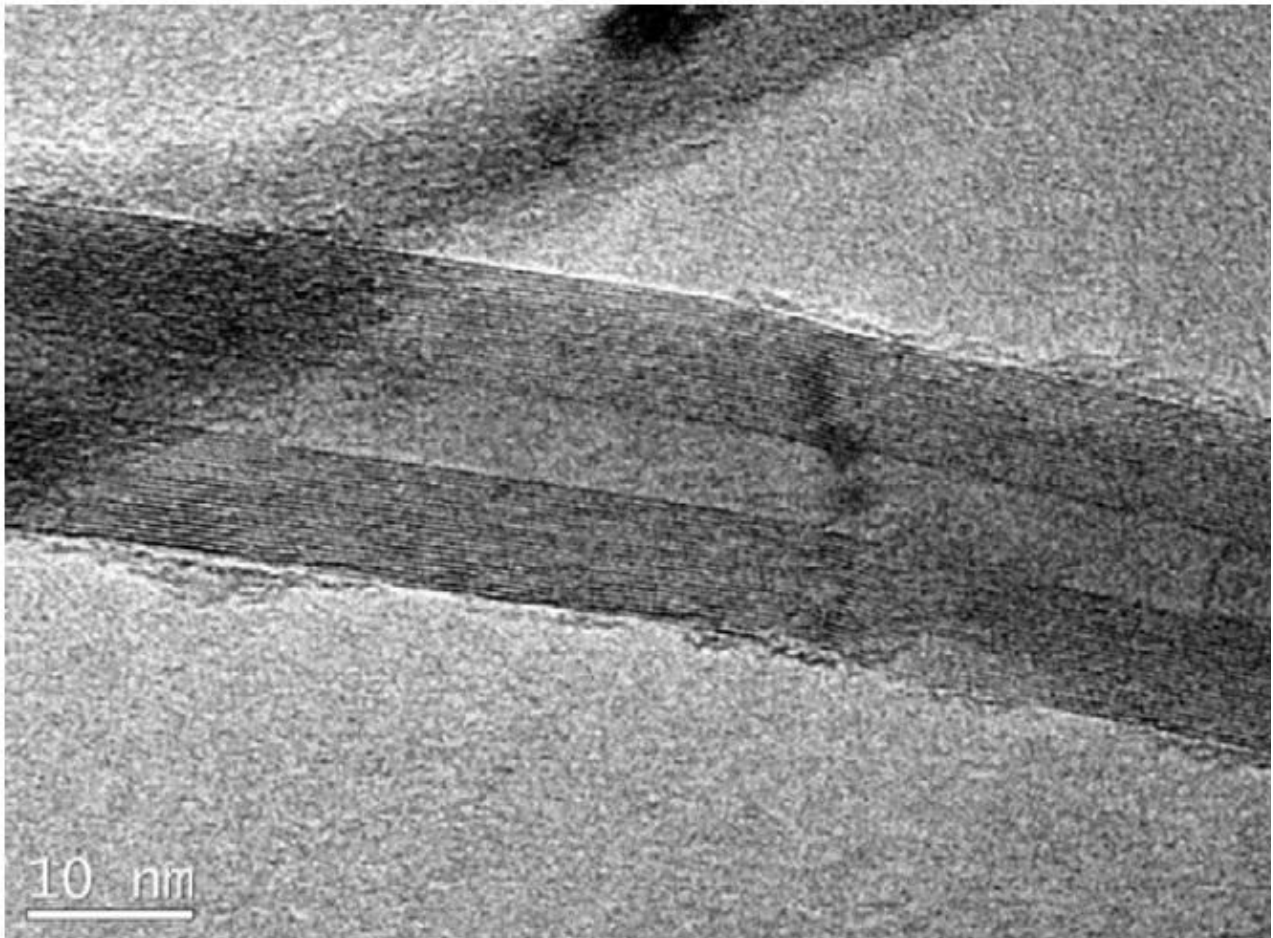
Results and discussions

Remarks on results incl. tables

A representative high resolution transmission electron micrograph of the MWCNT shows the distinctive crystalline structure of MWCNT, with this particular MWCNT having 20 walls.

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: TEM Crystalline phase.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies by reliable national institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Y. MORIMOTO, M. HIROHASHI, A. OGAMI, T. OYABU, T. MYOJO, M. TODOROKI, M. YAMAMOTO, M. HASHIBA, Y. MIZUGUCHI, B. WOO LEE, E. KURODA, M. SHIMADA, W. N. WANG, K. YAMAMOTO, K. FUJITA, S. ENDOH, K. UCHIDA, N. KOBAYASHI, K. MIZUNO, M. INADA, H. TAO, T. NAKAZATO, J. NAKANISHI, I. TANAKA	2012	Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation	Nanotoxicology Sep. 2012, Vol. 6, No. 6, Pages 587-599 (doi:10.3109/17435390.2011.594912)					

Materials and methods

Method

transmission electron microscopy (TEM)

Test materials

Test material identity

Identifier	Identity
	Nikkiso MWCNT

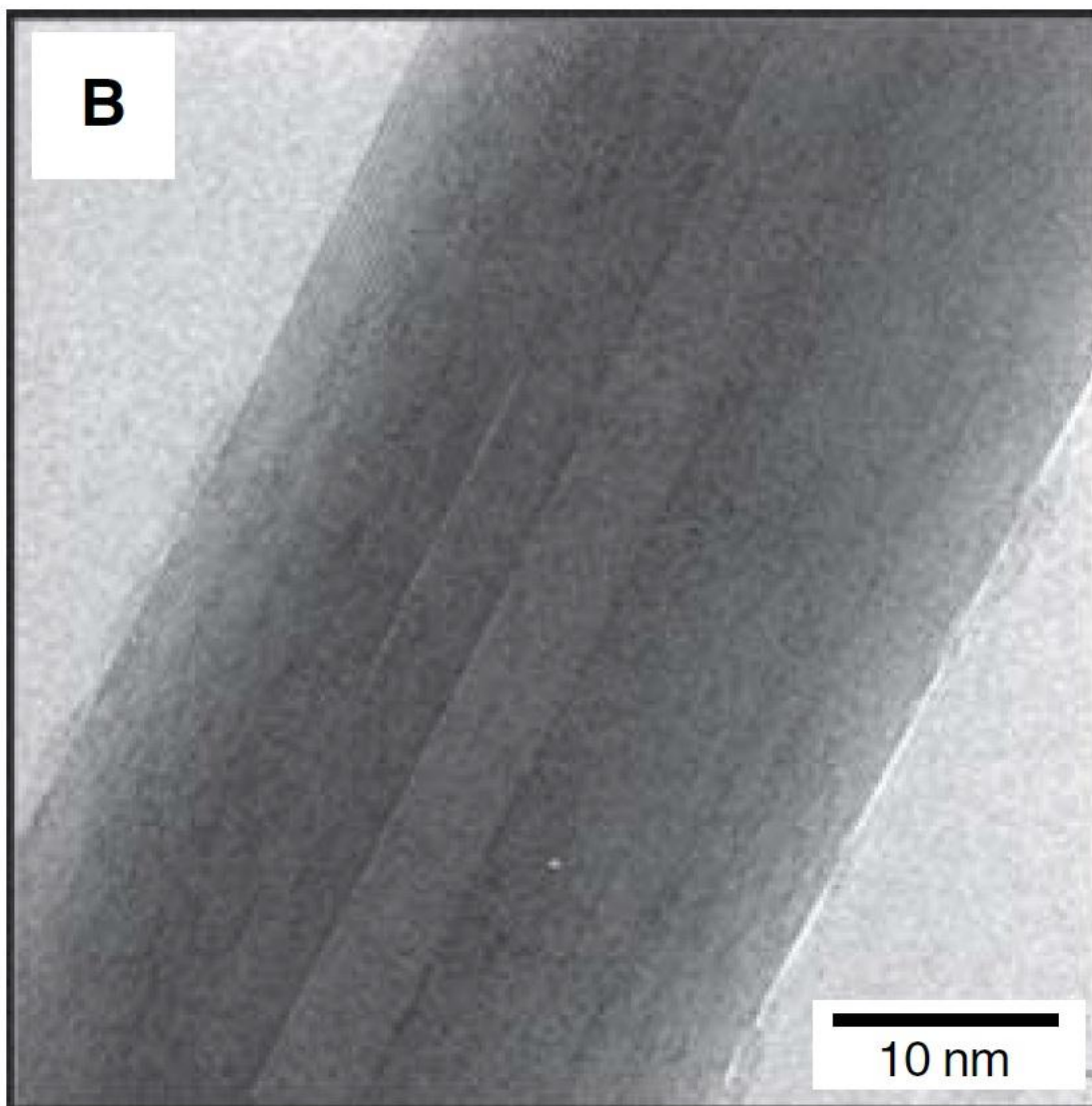
Results and discussions

Crystallographic composition

See TEM picture

Overall remarks, attachments

Illustration (picture/graph)



Endpoint study record: Raman Crystalline phase.002

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability deficiencies

incl. The experiment was carried out at the reliable national Institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST	2011	Risk assessment report of the nanomaterial - carbon nanotubes-	Nedo project (P06041)					

Materials and methods

Method

other: Raman Spectroscopy analysis

Test materials

Test material identity

Identifier	Identity
	Nikkiso MWCNT

Results and discussions

Remarks on results incl. tables

MWCNTs are in the structure of multiple rolled up of graphene sheets. The soundness of the structure (Defect of graphene sheet) can be seen by the ratio of G-band and D-band of Raman Spectrum.

G/D ratio = 8 +/- 3

Endpoint study record: Crystalline phase.001

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies reviewed paper

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	J. S. Kim, J. H. Sung, K. S. Song, J. H. Lee, S. M. Kim, G. H. Lee, K. H. Ahn, J. S. Lee, J. H. Shin, J. D. Park, I.J. Yu	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	Tox. Sci. 128 (2) 439-448 (2012)					

Materials and methods

Method

other: Raman Spectroscopy

Principles of method if other than guideline

Check the soundness of the crystal

Test materials

Test material identity

Identifier	Identity
	Hanwha MWCNT

Results and discussions

Crystallographic composition

The disorder and graphite peak of MWCNTs was observed at 1320 ~ 1330 cm⁻¹, 1570 ~ 1580 cm⁻¹. Based on peak intensity ratio (ID/IG), crystallizability of MWCNTs can be measured indirectly. ID/IG of grown MWCNTs was 1.238; this result represented MWCNTs contained high levels of defects

4.26 Crystallite and grain size

Endpoint study record: Crystallite and grain size.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

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									

Materials and methods

Method

transmission electron microscopy (TEM)

Test materials

Test material identity

Identifier	Identity
	Baytubes

Any other information on materials and methods incl. tables

MWCNT dispersed in H₂O/PEG with short ultrasonic treatment; Tube diameter distribution obtained by image analysis of TEM pictures (for each distribution ca. 200-400 tubes measured)

Results and discussions

Remarks on results including tables and figures

tube diameter d₅₀=11 nm

tube length 380-902 nm

Endpoint study record: Crystallite and grain size.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

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									

Materials and methods

Method

transmission electron microscopy (TEM)

Test materials

Test material identity

Identifier	Identity
	Arkema Graphistrength C100

Results and discussions

Remarks on results including tables and figures

Average internal diameter: 4.8 nm
Average external diameter: 11.7 nm
Average length: 1097 nm
Average number of walls: 10

Endpoint study record: Crystallite and grain size.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies measured at reliable institute (JBRC)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Asakura et al.	2010	Genotoxicity and Cytotoxicity of Multi-wall Carbon Nanotubes in Cultured Chinese Hamster Lung Cell in Comparison with Chrysolite A Fiber	J. Occup. Health 2010, 52, PP155-166					

Materials and methods

Method

other: SEM

Test materials

Test material identity

Identifier	Identity
	Mitui MWNT-7

Results and discussions

Remarks on results including tables and figures

length = 5.0 μm , (SD=4.5 μm)

Width = 88nm, (SD=5nm)

Endpoint study record: Crystallite and grain size.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

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									

Materials and methods

Method

transmission electron microscopy (TEM)

Test materials

Test material identity

Identifier	Identity
	Nanocyl NC7000

Results and discussions

Remarks on results including tables and figures

External diameter distribution: from 5 to 15 nm, with a mean value of 9 nm

Length distribution: from 0.1 to 10 μm with a mean value of 1.5 μm

Endpoint study record: Crystallite and grain size.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies The experiment was carried out at the reliable national Institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST	2011	Risk assessment report of the nanomaterial - carbon nanotubes-	Nedo Project (P06041)					

Materials and methods

Method

transmission electron microscopy (TEM)

Test materials

Test material identity

Identifier	Identity
	Nikkiso MWCNT

Results and discussions

Remarks on results including tables and figures

Dia = 48nm, (SD=1.1)
L = 0.94 μ m, (SD=2.3)
number of observation = 1,005

Endpoint study record: Crystallite and grain size.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies reviewed paper

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	J. S. Kim, J. H. Sung, K. S. Song, J. H. Lee, S. M. Kim, G. H. Lee, K. H. Ahn, J. S. Lee, J. H. Shin, J. D. Park, I.J. Yu	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	Tox. Sci. 128 (2) 439-448 (2012)					

Materials and methods

Method

other: SEM

Test materials

Test material identity

Identifier	Identity
	Hanwha MWCNT

Results and discussions

Remarks on results including tables and figures

Diameter: 10 to 15 nm

Length: less than 20 μ m

4.27 Aspect ratio/shape

4.28 Specific surface area

Endpoint study record: Specific surface area.001

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies According to DIN/ISO

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	J. 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	Tox. Sci 113 (1), 226-242 (2010)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	ISO 9277:2010 Determination of the specific surface area of solids by gas adsorption - BET method	

Test materials

Test material identity

Identifier	Identity
	Baytubes

Results and discussions

Specific Surface Area

Specific Surface Area ca. 253 m²/g

Endpoint study record: Specific surface area.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

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									

Materials and methods

Test materials

Test material identity

Identifier	Identity
	Arkema Graphistrength C100

Results and discussions

Specific Surface Area

Specific Surface Area 212 m²/g

Endpoint study record: Specific surface area.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies measured at reliable national institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Kobayashi et al.	2010	Biological response and morphological assessment of individually dispersed multi-wall carbon nanotubes in the lung after intratracheal instillation in rats	Toxicology 276 (2010), pp 143-153					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	ISO 9277:2010 Determination of the specific surface area of solids by gas adsorption - BET method	

Methods

BET

Test materials

Test material identity

Identifier	Identity
	Mitsui MWNT-7

Results and discussions

Specific Surface Area

Specific Surface Area ca. 23 m²/g

Endpoint study record: Specific surface area.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	ISO 9277:2010 Determination of the specific surface area of solids by gas adsorption - BET method	

Test materials

Test material identity

Identifier	Identity
	Nanocyl NC7000

Results and discussions

Specific Surface Area

Specific Surface Area ca. 230 m²/g

Endpoint study record: Specific surface area.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies The experiment was carried out at the reliable national Institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST	2011	Risk assessment report of the nanomaterial - carbon nanotubes-	Nedo Project (P06041)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	ISO 9277:2010 Determination of the specific surface area of solids by gas adsorption - BET method	

Methods

BET

Test materials

Test material identity

Identifier	Identity
	Nikkiso MWCNT

Results and discussions

Specific Surface Area

Specific Surface Area ca. 69.4 m²/g

Endpoint study record: Specific surface area.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies reviewed paper

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	J. S. Kim, J. H. Sung, K. S. Song, J. H. Lee, S. M. Kim, G. H. Lee, K. H. Ahn, J. S. Lee, J. H. Shin, J. D. Park, I.J. Yu	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	Tox. Sci. 128 (2) 439-448 (2012)					

Materials and methods

Methods

BET

Test materials

Test material identity

Identifier	Identity
	Hanwha MWCNT

Results and discussions

Specific Surface Area

Specific Surface Area ca. 224.9 m²/g

4.29 Zeta potential

Endpoint study record: Zeta potential.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

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									

Materials and methods

Test materials

Test material Identity

Identifier	Identity
	Baytubes

Any other information on materials and methods incl. tables

Zeta-potentials were determined at different pH values (2-11) with a Malvern Zetasizer 3000HSA at 25°C and averaged over 5 series of measurements

Results and discussions

Remarks on results incl. tables

The iso-electric point for Baytubes was found to be between pH 5-6.

Endpoint study record: Zeta potential.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The experiment was carried out at reliable national Institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST	2011	Risk assessment report of the nanomaterial - carbon nanotubes-	Nedo Project (P06041)					

Materials and methods

Details on methods and data evaluation

electrophoretic mobility of the sample materials was measured; and zeta potential was calculated based on the results.

Test materials

Test material Identity

Identifier	Identity
	Nikkiso MWCNT

Results and discussions

Zeta Potential

Zeta Potential ca. -14.7 mV

Standard Deviation 0.9

in medium DMEM-FBS10%

Endpoint study record: Zeta potential.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Information from Korean EPA									

Materials and methods

Test materials

Test material Identity

Identifier	Identity
	Hanwha MWCNT

Any other information on materials and methods incl. tables

ELS (electrophoretic light scattering) spectrometer, FT-IR

Results and discussions

Zeta Potential

Zeta > -45 — < -10 mV

Potential

Remarks on results incl. tables

In water phase, both M63 and M65 materials had zeta potential values ranging from -10mV ~ -45mV.

4.30 Surface chemistry

Endpoint study record: Surface chemistry.001

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

The experiment was carried out at the reliable national institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST	2011	Risk assessment report of the nanomaterial - carbon nanotubes-	Nedo Project (P06041)					

Materials and methods

Methods

XPS

Principles of method if other than guideline

This end @ point is not relevant to the principal MWCNT because the principal MWCNT is not surface treated. However, it is recommended to check the absence of functional groups and the defect density by, for example, determining the organic carbon content and the oxygen content by XPS.

Test materials

Test material Identity

Identifier	Identity
	Nikkiso MWCNT

Results and discussions

Functionalisation

Remarks on results incl. tables

O/C = 0.004

4.31 Dustiness

Endpoint study record: Dustiness.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EN 15051: 2006 (Workplace atmospheres - Measurement of the dustiness of bulk materials - Requirements and reference test methods)	

Methods

continuous drop

Test materials

Test material Identity

Identifier	Identity
	Baytubes

Results and discussions

Remarks on results incl. tables

The respirable dustiness was low.

Endpoint study record: Dustiness.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Reviewd article

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	L. Ma-Hock, S. Treumann, V. Strauss, S. Brill, F. Luizi, M. Mertler, K. Wiench, A. O. Gamer, B. Ravenzwaay, R. Landsiedel	2009	Inhalation Toxicity of Multiwall Carbon Nanotubes in Rats Exposed for 3 Months	TOXICOLOGICAL SCIENCES 112(2), 468-481 (2009)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: DIN 55992-2	

Methods

other: drop

Test materials

Test material Identity

Identifier	Identity
	Nanocyl NC7000

Results and discussions

Remarks on results incl. tables

The dustiness measurements (DIN 55992-2, Drop method) detected no nanoscale particles above ambient levels, and only a small number of microscale agglomerates. This lack of respirable dust is consistent with the macroscopic appearance of the test substance.

Endpoint study record: Dustiness.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	The experiment was carried out reliable national Institute (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST		Risk assessment report of the nanomaterial - carbon nanotubes-	Nedo Project (P06041)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Methods

vortex shaker

Test materials

Test material Identity

Identifier	Identity
	Nikkiso MWCNT

Any other information on materials and methods incl. tables

The test material in a range of 0.5 to 1 cm³ are placed in a glass test tube. They are agitated using a laboratory vortex shaker. The number concentrations and size distributions of aerosol particles released during agitation are continuously measured using an APS (Aerodynamic Particle Sizer), an OPC (Optical Particle Counter), a SMPS (Scanning Mobility Particle Sizer) and a CPC (Condensation Particle Counter).

Results and discussions

Remarks on results incl. tables

Respirable mass conc. = 0.061 mg/m³

4.32 Porosity

Endpoint study record: Porosity.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies According to DIN/ISO

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	J. 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	Tox. Sci 113 (1), 226-242 (2010)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	ISO 15901-1:2005 with Cor 1:2007 (Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption - Part 1: Mercury porosimetry)	

Test materials

Test material Identity

Identifier	Identity
	Baytubes

Any other information on materials and methods incl. tables

The agglomerate density of Baytubes were measured using the Hg-porosimetry method. Instrument: PASCAL 140/440 from Thermo Electron Corp. Mercury pressure of 3760 mbar (equivalent to 4000 nm pore width), corresponding to the inflection point of the cumulative pore volume curve between inter-particle volume filling and compaction of the agglomerate (surface tension 480 dny/cm; mercury contact angle 141°, room temperature).

Results and discussions

Porosity (fraction of void space in the material)

Mean ca. 0.32 g/cm³

Endpoint study record: Porosity.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to JIS/ISO

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	JFE Techno-Research	2011	Study on the current domestic and foreign risk assessment and the information on risk of nanomaterials	METI Study Report	SECAS				

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	ISO 15901-1:2005 with Cor 1:2007 (Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption - Part 1: Mercury porosimetry)	

Test materials

Test material Identity

Identifier	Identity
	Nikkiso MWCNT

Results and discussions

Specific pore volume

Mean ca. 24.6 cm³/g

Remarks on results incl. tables

Pore volume: 24.6 ml/g,
D_{avg} = 420 nm

4.33 Pour density

Endpoint study record: Pour density.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Following DIN/ISO

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	J. 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	Tox. Sci 113 (1), 226-242 (2010)					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: DIN/ISO 9136	

Test materials

Test material Identity

Identifier	Identity
	Baytubes

Results and discussions

Pour Density

Mean ca. 0.16 g/cm³

Endpoint study record: Pour density.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability other:

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									

Materials and methods

Method

other: Ca MDA 053-mercury porosity

Test materials

Test material Identity

Identifier	Identity
	Arkema Graphistrength C100

Results and discussions

Pour Density

Mean 0.09 g/cm³

Endpoint study record: Pour density.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 4 (not assignable)

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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: DIN ISO 9136	

Test materials

Test material Identity

Identifier	Identity
	Nanocyl NC7000

Results and discussions

Pour Density

Mean ca. 0.06 g/cm³

Endpoint study record: Pour density.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies According to ASTM D

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	JFE Techno-Research	2011	Study on the current domestic and foreign risk assessment and the information on risk of nanomaterials	METI Study Report					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: ASTM D 1513-05	

Method

continuous drop

Principles of method if other than guideline

The test material was poured into a 1 little cylindrical container from the height not more than 50 mm without settling.

Test materials

Test material Identity

Identifier	Identity
	Nikkiso MWCNT

Results and discussions

Pour Density

Mean ca. 3.8 g/cm³

4.34 Photocatalytic activity

Endpoint study record: Photocatalytic activity.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	According to ISO/JIS guidance

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	JFE Techno-Research	2011	Study on the current domestic and foreign risk assessment and the information on risk of nanomaterials	METI Study Report					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: ISO 22197-1 / JIS R 1701	

Test materials

Test material Identity

Identifier	Identity
	Nikkiso MWCNT

Any other information on materials and methods incl. tables

Decomposition of acetaldehyde were examined in the closed chamber under UV radiation.

Results and discussions

Photocatalytic Activity equation/description

No photocatalytic activity

Remarks on results incl. tables

Removal rate of acetaldehyde and conversion rate to carbon dioxide

Post UV radiation time (hrs)	Materials					
	Nikkiso MWCNT		Titanium oxide (Positive control)		Iron powder (Negative control)	
	CH ₃ CHO	CO ₂	CH ₃ CHO	CO ₂	CH ₃ CHO	CO ₂
0.5	≤5%	≤5%	63.3%	27.3%	≤5%	≤5%
1.0	≤5%	≤5%	93.3%	56.8%	≤5%	≤5%
1.5	≤5%	≤5%	≥95%	76.6%	≤5%	≤5%
2.0	≤5%	≤5%	≥95%	90.1%	≤5%	≤5%

5. ENVIRONMENTAL FATE AND PATHWAYS

5.1 Stability

5.2 Biodegradation

5.2.1 Biodegradation in water: screening tests

Endpoint study record: Baytubes Biodegradation TG301F

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									

Materials and methods

Test type

ready biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test)	

GLP compliance

no data

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Baytubes

Study design

Duration of test (contact time)

ca. 28 d

Results and discussions

Details on results

28d, 0%

Applicant's summary and conclusion

Interpretation of results

under test conditions no biodegradation observed

Conclusions

not readily biodegradable

Endpoint study record: Graphistrength C100 Biodegradation TG301F

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	According to OECD TG

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									

Materials and methods

Test type

ready biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test)	

GLP compliance

no data

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Results and discussions

BOD5 / COD results

Any other information on results incl. tables

As expected, no “mineralization” was detected by consumption of oxygen by inoculum. All ready aerobic biodegradability methods are designed for measurement of oxidation of organic substances (that is, by definition, substances containing C-H bonds) which CNTs are not.

Endpoint study record: Nikkiso MWCNT Biodegradation 301C

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			CERI	15621			

Materials and methods

Test type

ready biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 301 C (Ready Biodegradability: Modified MITI Test (I))	

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

Study design

Inoculum or test system

activated sludge, non-adapted

Duration of test (contact time)

ca. 28 d

Initial test substance concentration

Initial conc.	Based on
ca. 100 mg/L	

Details on study design

Bottle 1: test substance in water
Bottles 2, 3 and 4: test substance and inoculums (activated sludge) in mineral medium
Bottle 5: reference substance (aniline) and inoculum in mineral medium
Bottle 6: inoculum in mineral medium
Temperature: 25±1°C, under dark, stirring during test period (28 days)

Reference substance

aniline

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 62 % and 73 % (Aniline, after 7 days and 14 days, respectively)DOC due to test substance: 0 mg-C/L

BOD5 / COD results

Any other information on results incl. tables

BOD and DOC in 28 days

	Water + Test material	Sludge + Test material			Theoretic value
Sample No.	(1)	(2)	(3)	(4)	--
BOD (mg)	0.7	-0.1	-0.4	-1.6	79.8
DOC (mg-C)	0	0	-0.5	-0.5	30
DOC detect ratio (%)	0	0	-2	-2	--

Degradation (BOD) in 28 days

	Sludge + Test sample			
Sample No.	(2)	(3)	(4)	average
Degradation (%)	0	-1	-2	0(*)

Change in BOD

Sample No.	Sample description	BOD (mg)			
		7 th day	14 th day	21 st day	28 th day
(1)	Water + test material	0.0	0.0	0.0	0.7
(2)	Sludge + test material	0.8	3.2	5.4	7.3
(3)	Sludge + test material	0.9	2.9	5.1	7.0
(4)	Sludge + test material	0.8	2.7	4.1	5.8
(5)	Control (blank)	1.2	4.2	5.6	7.4
(6)	Control (Sludge + aniline)	57.3	70.5	74.5	77.5

Overall remarks, attachments

Illustration (picture/graph)

Study No. 15621	(Test item 物質B)
Cultivation conditions:	
Concentration	
Test item	30 (mg/L)
Reference item aniline.....	100 (mg/L)
Activated sludge	30 (mg/L)
Temperature	25 ± 1 °C
Duration	28days (Jan.24,2011 - Feb.21,2011)
Note: —	

Vessel No.	Sample Description	BOD (mg)			
		7th day	14th day	21st day	28th day
[1]	Water + test item	0.0	0.0	0.0	0.7
[2]	Sludge + test item	0.8	3.2	5.4	7.3
[3]	Sludge + test item	0.9	2.9	5.1	7.0
[4]	Sludge + test item	0.8	2.7	4.1	5.8
[5]	Control blank [B]	1.2	4.2	5.6	7.4
[6]	Sludge + aniline	57.3	70.5	74.5	77.5

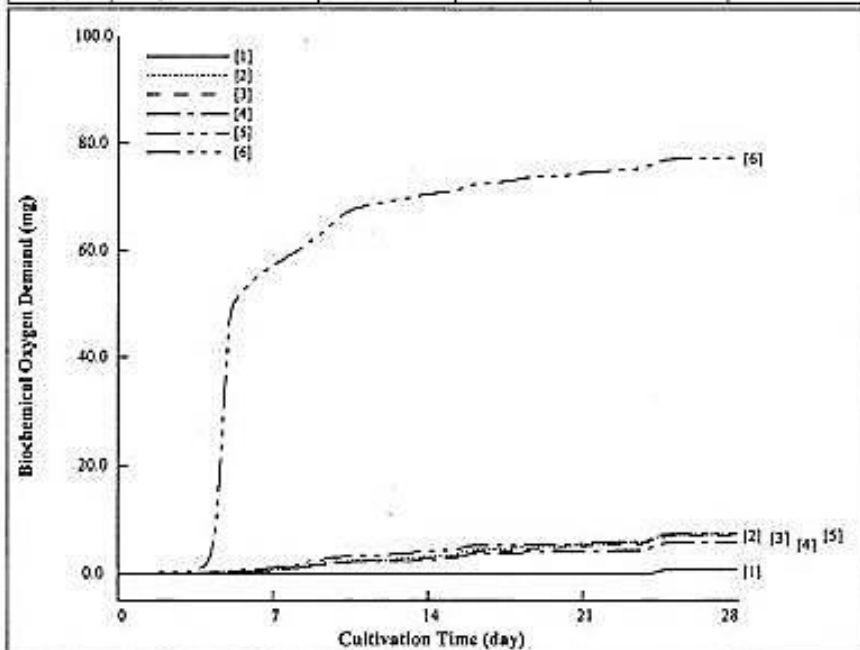


Fig. 1 Chart of BOD.

Applicant's summary and conclusion

Interpretation of results

under test conditions no biodegradation observed

Conclusions

The result indicated that the test substance is not readily biodegradable.

Endpoint study record: Nikkiso MWCNT Biodegradation TG 301F

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			CERI	15605			

Materials and methods

Test type

ready biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test)	

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Nikkiso MWCNT

Study design

Inoculum or test system

activated sludge, adapted

Duration of test (contact time)

>= 28 d

Initial test substance concentration

Initial conc.	Based on
>= 100 mg/L	test mat.
>= 30 mg/L	act. ingr.

Details on study design

Bottles 1 & 2: containing test substance and inoculums (activated sludge) [test suspension]

Bottles 3 & 4: containing only inoculum [inoculum blank] Bottle 5: containing reference compound (aniline) and inoculum [procedure control]

Temperature: 22 ± 1 °C, under dark, stirring during test period (28 days)

Reference substance

aniline

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 84 % (Aniline, after 14 days)

DOC due to test substance: 0 mg-C/L

BOD5 / COD results

Any other information on results incl. tables

BOD and DOC in 28 days

Sample No.	Sludge + Test material		Sludge only		Theoretic value
	(1)	(2)	(3)	(4)	
BOD (mg)	-4.4	-2.6	7.7	8.1	79.8
DOC (mg-C/L)	0.4	0.3	0.4	0.3	30
DOC detect ratio (%)	0	0	--	--	--

Degradation (BOD) in 28 days

Sample No.	Sludge + Test sample		
	(2)	(3)	average
Degradation (%)	-6	-3	0(*)

(*) The average value became negative, so the value set as 0

Change in BOD

Sample No.	Sample description	BOD (mg)			
		7 th day	14 th day	21 st day	28 th day
(1)	Sludge + test material	2.0	2.9	2.9	3.5
(2)	Sludge + test material	3.1	4.4	4.4	5.3
(3)	Control (blank)	3.6	5.2	6.9	7.7
(4)	Control (blank)	3.7	5.4	7.3	8.1
(5)	Control (Sludge + aniline)	56.8	81	86.3	88.4

Overall remarks, attachments

Illustration (picture/graph)

Study No. 15605	(Test item 供試試料A)
Cultivation conditions:	
Concentration	
Test item	100 (mg/L)
Reference item aniline	100 (mg/L)
Activated sludge	30 (mg/L)
Temperature	22 ± 1 °C
Duration	28days (Dec.14,2010 - Jan.11,2011)
Note:	

Vessel No.	Sample Description	BOD (mg)			
		7th day	14th day	21st day	28th day
[1]	Sludge + test item	2.0	2.9	2.9	3.5
[2]	Sludge + test item	3.1	4.4	4.4	5.3
[3]	Control blank [B]	3.6	5.2	6.9	7.7
[4]	Control blank [B]	3.7	5.4	7.3	8.1
[5]	Sludge + aniline	56.8	81.0	86.3	88.4

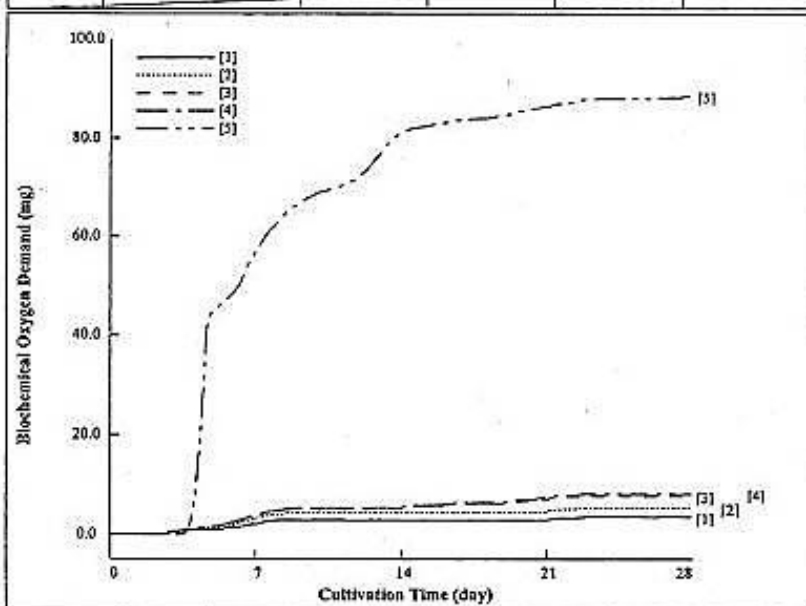


Fig. 1 Chart of BOD.

Applicant's summary and conclusion

Interpretation of results

under test conditions no biodegradation observed

Conclusions

The result indicated that the test substance is not readily biodegradable

Endpoint study record: Nikkiso MWCNT Biodegradation TG302C

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			CERI	15622			

Materials and methods

Test type

inherent biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II))	

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

Study design

Inoculum or test system

activated sludge, adapted

Duration of test (contact time)

>= 28 d

Initial test substance concentration

Initial conc.	Based on
>= 30 mg/L	test mat.
>= 100 mg/L	act. ingr.

Parameter followed for biodegradation estimation

DOC removal
other: BOD

Details on study design

Bottle 1: test substance in water

Bottles 2, 3 and 4: test substance and inoculums (activated sludge) in mineral medium

Bottle 5: reference compound (aniline) and inoculum in mineral medium

Bottle 6: inoculum in mineral medium

Temperature: 25±1oC , under dark, stirring during test period (28 days)

Reference substance

aniline

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 1 % (Test substance, after 28 days), 59 % and 74 % (Aniline, after 7 days and 14 days, respectively)DOC due to test substance: 0 mg-C/L

Overall remarks, attachments

Remarks on results including tables and figures

BOD and DOC in 28 days

Sample No.	Water + Test material	Sludge + Test material			Theoretic value
	(1)	(2)	(3)	(4)	
BOD (mg)	2.2	1.1	1.5	-1.6	23.9
DOC (mg-C/L)	0	0	-0.2	0	9
DOC detect ratio (%)	0	0	-2	0	--

Degradation (BOD) in 28 days

Sample No.	Sludge + Test sample			average
	(2)	(3)	(4)	
Degradation (%)	5	6	-7	1

Change in BOD

Sample No.	Sample description	BOD (mg)			
		7 th day	14 th day	21 st day	28 th day
(1)	Water + test material	0.0	0.0	1.3	2.2
(2)	Sludge + test material	6.2	14.0	21.0	22.5
(3)	Sludge + test material	7.2	15.4	20.6	22.9
(4)	Sludge + test material	6.3	12.9	17.4	19.8
(5)	Control (blank)	5.9	13.3	18.5	21.4
(6)	Control (Sludge + aniline)	55.3	70.4	75.1	77.9

Illustration (picture/graph)

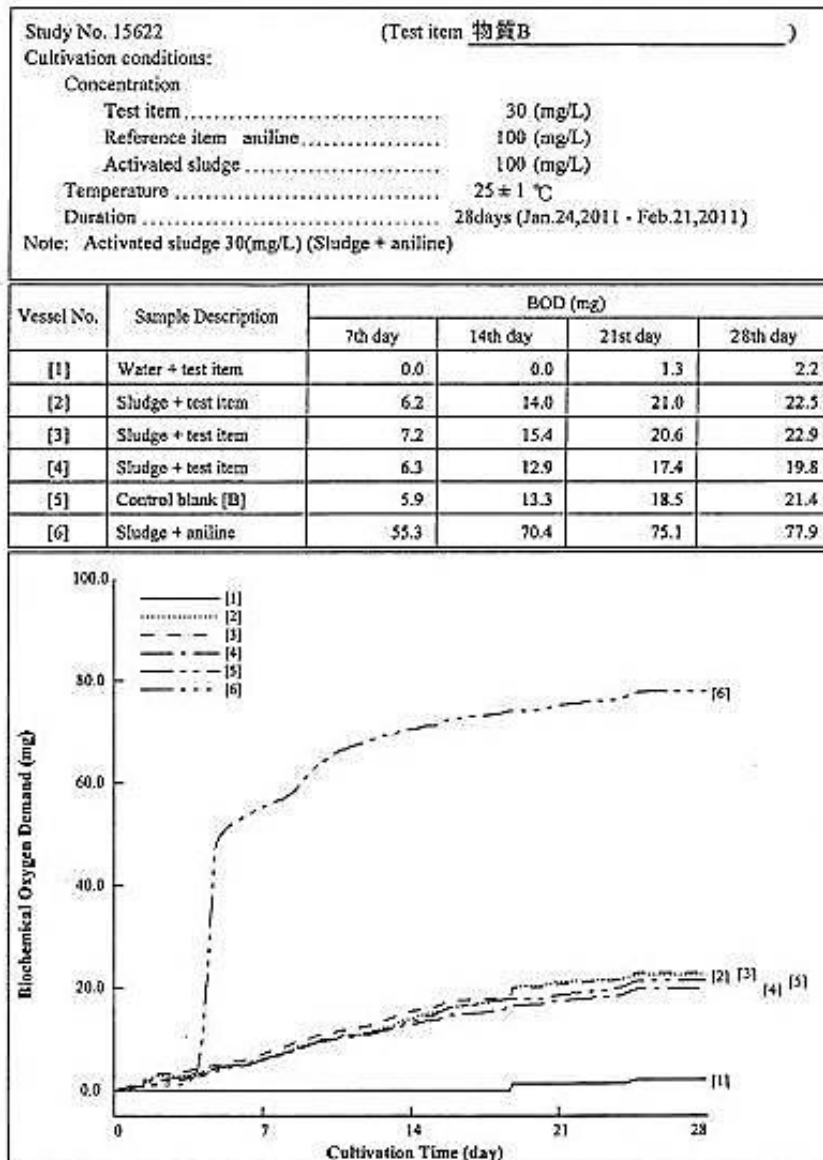


Fig. 1 Chart of BOD.

Applicant's summary and conclusion

Interpretation of results

under test conditions no biodegradation observed

Conclusions

The result indicated that the test substance is not biodegradable

5.2.2 Biodegradation in water and sediment: simulation tests

5.2.3 Biodegradation in soil

5.2.4 Mode of degradation in actual use

5.3 Bioaccumulation

5.4 Transport and distribution

5.5 Environmental data

5.6 Additional information on environmental fate and behaviour

6. ECOTOXICOLOGICAL INFORMATION

6.1 Aquatic toxicity

6.1.1 Short-term toxicity to fish

Endpoint study record: Baytubes (TG 203) Short-term toxicity to fish.002

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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 203 (Fish, Acute Toxicity Test)	

GLP compliance

no data

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Baytubes

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 100 mg/L				

Endpoint study record: Graphistrength C100 (TG 203) Short-term toxicity to fish.001

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies Following GLP and OECD TG

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2010		REACH Dossier; Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					2010-04-04

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 203 (Fish, Acute Toxicity Test)	

GLP compliance

yes (incl. certificate)

Test materials

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

yes

Test material identity

Identifier	Identity
	Arkema Graphistrength C100

Analytical monitoring

yes

Details on sampling

- Concentrations: Range finding test: 1, 35, 50 and 100 mg/L in dilution water

Definitive test: limit test at 100 mg/L

- Sampling method:

For the range finding test:

Suspensions of GRAPHISTRENGTH C100 were directly prepared for each concentration by weighing the convenient amounts of test item into 100 mL of dilution water and adjusting to 5 L in the test tanks.

Final tested concentrations were as follow: 1, 35, 50 and 100 mg/L.

For the definitive test: The 100 mg/L suspension of GRAPHISTRENGTH C100 was directly prepared by weighing the convenient amount of test item into 100 mL of dilution water and adjusting it to 5 L in the test tanks.

- Sample storage conditions before analysis: an axial propeller stirring device is used in order to maintain GRAPHISTRENGTH C100 in suspension.

Vehicle

yes

Details on test solutions

PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances)

- Method: The stock solution was prepared by dispersing the test item in reconstituted water made up by diluting pure mineral salts (analytical grade) in ultra-pure water according to the composition described in the OECD 203 Guideline. The test solutions were then prepared by diluting the stock solutions in reconstituted water.

Test organisms

Test organisms (species)

Danio rerio

Details on test organisms

- Strain: Danio rerio (Teleostei, Cyprinidae).

- Source: Aquatrade France (44150 Ancenis).

- Age at study initiation (mean and range, SD): date of birth stated by the supplier: 29/09/09
- Length at study initiation (length definition, mean, range and SD): (determined by measuring 10 randomly sampled fish), 3.2 ± 0.3 cm.
- Method of breeding: no data
- Feeding during test: no data

ACCLIMATION

- Acclimation period: The fish were placed under the test conditions (medium, identical or lower biological loading, temperature, lighting, etc.) 20 days before the beginning of the test.

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

96 h

Test conditions

Hardness

According to the standard medium: CaCl₂, 2H₂O: 11.76 g per litre of water, MERCK Ref. 1.02382.5000 batch A935182902 MgSO₄, 7H₂O: 4.93 g per litre of water, MERCK Ref. 1.05886.1000 batch A975086904 NaHCO₃: 2.59 g per litre of water, MERCK Ref. 1.06329.0500 batch K39347429847 KCl: 0.23 g of per litre of water, MERCK Ref. 1.04936.0250 batch K37429936851

Test temperature

The temperature, which was measured on a continuous basis in a test flask, was $20.1 \pm 1^\circ\text{C}$.

pH

pH between 7.46 and 7.87

Dissolved oxygen

Dissolved oxygen between 93 and 99 %

Salinity

Fresh water test

Nominal and measured concentrations

The highest tested concentration 100 mg/L

Details on test conditions

The study made use of no dispersing agents to stabilize dispersion of MWNCTs in the water column but rather used mechanical stirring in an effort to keep CNTs in suspension.

Reference substance (positive control)

yes (Potassium dichromate)

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 100 mg/L	nominal	test mat.	mortality	

Details on results

- Behavioural abnormalities: no- Mortality of control: no

Results with reference substance (positive control)

LC50-24h=250 mg/L

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Validity criteria:

- The mortality in the control did not exceed 10 per cent at the end of the test;
- The concentration of dissolved oxygen in the test vessels remained above 60 % of the air saturation value at the end of the test;

Deviations from the guideline: none

- The pH did not vary by more than 1 unit

Executive summary

The determination of the mortality of fish exposed to GRAPHISTRENGTH C100 for a duration of 96 hours was assessed according to the OECD Guideline 203. Fish were exposed under static conditions to 100 mg/L of GRAPHISTRENGTH C100 dispersed in dilution water (limit test). The concentrations of test item causing a 50 % mortality at 24-, 48-, 72- and 96-h (LC₅₀) were estimated. The results are as follows: LC50 -24h, LC50 -48h, LC50 -72h and LC50 -96h determined are > 100 mg/L. Furthermore, no mortality was observed at any tested concentration. LC0 -96h > 100 mg/L

Endpoint study record: Nanocyl NC7000 (TG204) Long-term toxicity to fish.001

Administrative Data

Purpose flag key 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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 204 (Fish, Prolonged Toxicity Test: 14-day 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:	Nanocyl NC7000

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
	LOEC	> 100 mg/L				

Endpoint study record: Nanocyl NC7000 (TG212) Long-term toxicity to fish.002

Administrative Data

Purpose flag key 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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 212 (Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages)	

Test materials

Test material identity

Identifier	Identity
other:	Nanocyl NC7000

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
	LOEC	> 100 mg/L				

Endpoint study record: Nikkiso MWCNT (TG 203) Short-term toxicity to fish.001

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			CERI	95399			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 203 (Fish, Acute Toxicity Test)	

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Nikkiso MWCNT

Test organisms

Test organisms (species)

Oryzias latipes

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

96 h

Test conditions

Hardness

27 mg/L (as CaCO₃)

Test temperature

24+/-1 C

pH

7.3

Nominal and measured concentrations

Test substance: 10 mg/L , Dispersing agent (HCO-40 (ethylene oxide adduct of hydrogenated castor oil): 100 mg/L

Details on test conditions

Static test with moderate aeration, No change of solution Exposure duration: 96 hours, Light: 16 hours photoperiod daily, Feed: none

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	> 10 mg/L	nominal	test mat.	mortality	

Any other information on results incl. tables**Cumulative mortality**

Nominal concentration (mg/L)	Cumulative mortality (%)				
	3 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
Vehicle control	0	0	0	0	0
10	0	0	0	0	0

Observed abnormal response

Nominal concentration (mg/L)	Result of observation (Left column: Number of affected fish/Total survival number Right column: Symptom detail)									
	3 hours		24 hours		48 hours		72 hours		96 hours	
	Control	0/7	N	0/7	N	0/7	N	0/7	N	0/7
Vehicle control	0/7	N	0/7	N	0/7	N	0/7	N	0/7	N
10	0/7	N	0/7	N	0/7	N	0/7	N	0/7	N

6.1.2 Long-term toxicity to fish

Endpoint study record: Nikkiso MWCNT (TG 204) Long-term toxicity to fish.001

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			Mitsubishi Chemical Medience	A100701			

Materials and methods

Life stage / endpoint studied

adult fish: (sub)lethal effects

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 204 (Fish, Prolonged Toxicity Test: 14-day Study)	

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

Vehicle

yes

Details on test solutions

Test substance: 0.10, 0.32, 1.0, 3.2, 10 mg/L, Dispersing agent: 100 mg/L (Tween 80)

Test organisms

Test organisms (species)

Oryzias latipes

Study design

Test type

semi-static

Water media type

freshwater

Limit test

yes

Total exposure duration

14 d

Test conditions

Test temperature

24+/-1 C

pH

7.5

Nominal and measured concentrations

Dose/concentration level: Test substance Conc.1; 0.10, Conc.2; 0.32, conc3; 1.0, Conc4; 3.2 and Conc5; 10 mg/L Control: Control (no test substance and dispersing agent) , Vehicle control (dispersing agent only)

Details on test conditions

Test Item: Lethal effects and effects other than lethal effects Number of species per sex per dose: Ten fishes in each test, control or vehicle control solution Semi-static test with moderate aeration, Change of solution: 3 times/week Exposure duration: 14 days, Light: 16 hours photoperiod daily, Temperature: 24+/-1 C

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
14 d	NOEC	3.2 mg/L	nominal	test mat.	behaviour	
14 d	LOEC	10 mg/L	nominal	test mat.	behaviour	
14 d	LC50	> 10 mg/L	nominal	test mat.	mortality	

Details on results

Threshold level of lethal effect: 10 mg/L

Any other information on results incl. tables

Number of survival and dead Fish

Test group	Number of survival and dead Fish														Mortality (%)	
	Day	1	2	3	4	5	6	7	8	9	10	11	12	13		14
Control	Survival	10	10	10	10	10	10	10	10	10	9	9	9	9	9	10
	Death	0	0	0	0	0	0	0	0	0	1	1	1	1	1	
Solvent control	Survival	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0
	Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0.10 mg/L	Survival	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0
	Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0.32 mg/L	Survival	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0
	Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1.0 mg/L	Survival	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0
	Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3.2 mg/L	Survival	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0
	Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10 mg/L	Survival	10	9	9	9	9	9	9	9	9	9	9	9	9	9	10
	Death	0	1	1	1	1	1	1	1	1	1	1	1	1	1	

Observation of Abnormal Symptoms

Test group	Number of survival and dead Fish					
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14
Control	N	N	N	N	N	N
Sol. control	N	N	N	N	N	N
0.10 mg/L	N	N	N	N	N	N
0.32 mg/L	N	N	N	N	N	N
1.0 mg/L	N	N	N	N	N	N
3.2 mg/L	N	N	N	N	N	N
10 mg/L	N	N	N	N	N	N

Observation of Feeding Activity

Test group	Feeding Activity					
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14
Control	N	N	N	N	N	N
Sol. control	N	N	N	N	N	N
0.10 mg/L	N	N	N	N	N	N
0.32 mg/L	N	N	N	N	N	N
1.0 mg/L	N	N	N	N	N	N
3.2 mg/L	N	N	N	N	N	N
10 mg/L	N	N	N	N	N	N

Wet Body Weight of Survival Fish at the End of Exposure

	Control	Sol. Control	0.10 mg/L	0.32 mg/L	1.0 mg/L	3.2 mg/L	10 mg/L
Wet body weight (mg)	162	195	248	212	181	170	117
	134	172	181	226	198	183	195
	137	166	176	184	180	155	142
	162	194	138	260	176	139	171
	102	143	136	233	214	164	156
	87	110	139	127	174	105	86
	64	158	117	183	223	105	88
	98	133	144	178	117	127	87
	69	89	96	165	215	128	57
	-	89	118	218	186	103	-
N	9	10	10	10	10	10	9
Total Mean	113	145	149	199	186	13.8	122
Total S. D.	37.4	39.3	43.2	38.6	30.2	29.1	46.4
Total C. V.	33.2	27.1	28.9	19.4	16.2	21.1	38

Total Body Length of Survival Fish at the End of Exposure

	Control	Sol. Control	0.10 mg/L	0.32 mg/L	1.0 mg/L	3.2 mg/L	10 mg/L
Wet body weight (mg)	24.2	27.0	28.0	24.8	21.9	25.2	21.6
	22.7	24.5	26.0	23.6	23.6	25.3	26.6
	24.9	25.9	25.8	22.8	22.4	24.1	20.7
	24.2	27.0	22.4	24.7	23.5	23.8	24.4
	20.9	24.4	22.0	25.2	25.6	23.7	24.8
	21.8	23.2	23.8	22.2	24.1	23.1	21.0
	18.9	22.2	23.4	24.0	24.7	21.6	21.8
	21.3	22.9	25.7	23.6	20.2	20.6	20.0
	18.3	20.2	20.4	25.0	24.5	20.8	17.9
	-	18.4	21.9	24.7	23.0	19.6	-
N	9	10	10	10	10	10	9
Total Mean	22.1	23.6	23.9	24.1	23.4	22.8	22.1
Total S. D.	2.36	2.81	2.37	1.00	1.56	2.00	2.7
Total C.V.	10.7	11.9	9.9	4.1	6.7	8.8	12.2

Overall remarks, attachments

Illustration (picture/graph)

Table 10 Threshold Level of Lethal Effect, Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) at the End of Exposure

Exposure period (days)	Threshold level of lethal effect (mg/L)	LOEC (mg/L)	NOEC (mg/L)
14	10	10	3.2

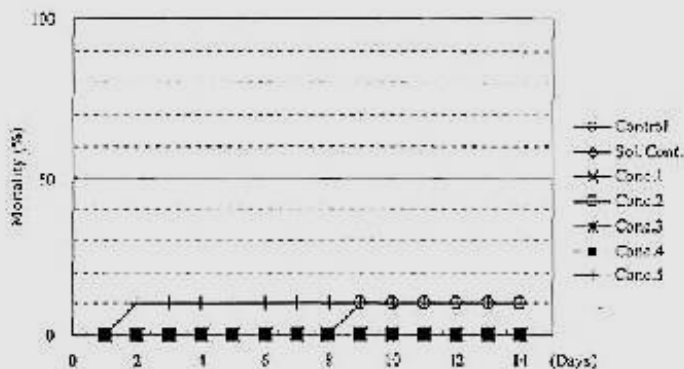
Table 11 Median Lethal Concentrations at the End of Exposure

Exposure Period (Days)	LC50 (mg/L)	95 Percent Confidence Limits (mg/L)	Statistical Method
14	>10 ^a	—	—

^a Not available

* The LC50 value and its 95% confidence limits could not be determined by statistical method because the mortality of fish at the maximum concentration level was less than 50%. Therefore, the LC50 value was given as an estimated concentration range.

Figure 1 Cumulative Mortality during Exposure



6.1.3 Short-term toxicity to aquatic invertebrates

Endpoint study record: Baytubes (TG 202) Short-term toxicity to aquatic invertebrates.003

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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	

Test materials

Test material identity

Identifier	Identity
other:	Baytubes

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC50	> 100 mg/L				

Endpoint study record: Graphistrength C100 (TG 202) Short-term toxicity to aquatic invertebrates.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
study report		2010		REACH Dossier; Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					2010-03-28

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	no

GLP compliance

yes (incl. certificate)

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:	Graphistrength C100

Analytical monitoring

yes

Details on sampling

- Concentrations: For the preliminary test, a series of 6 concentrations (100, 50, 10, 5, 1 and 0.1 mg/L) of the test item in dilution water.

Concerning the definitive test, based on the results of preliminary test, it was decided to perform a limit test at 100mg/L.

- Sampling method

Preliminary test: In the preliminary test, daphnids were exposed under static conditions to a series of 6 concentrations of GRAPHISTRENGTH C100 in dilution water. The toxic effect measured during the assay was the inhibition mobility over a time period of 48 hours. Approximately 90% of the final volume of the test suspensions was added into test flasks. Five *Daphnia magna* aged from 6 to 24 hours were added into each test flask, which were then totally filled with the test suspension (30 mL). Two replicates were prepared for each concentration.

.Definitive test: Based on the results of preliminary test, it was decided to perform a lirait test at 100 mg/L. Volumetric flasks were filled with the required volumes of the test item stock suspension.

Approximately 90% of the final volume of the test suspension was added into test flasks

- Sample storage conditions before analysis: In order to keep GRAPHISTRENGTH C100 in suspension, flasks were continuously maintained under axial rotation by use of a cylindrical roller device

Vehicle

yes

Details on test solutions

The study made use of no dispersing agents to stabilize dispersion of MWNCTs in the water column but rather used mechanical stirring in an effort to keep CNTs in suspension.

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

- Common name:

- Strain: *Daphnia magna* Straus

- Source: from a stock breeding in the laboratory reared in VOLVIC water added with vitamin B12, Na₂SeO₃, 5H₂O, calcium and magnesium salts. Selection of test organisms less than 24 hours-old is made by filtration.

- Age at study initiation (mean and range, SD): from 6 to 24 hours

- Weight at study initiation (mean and range, SD): no data- Length at study initiation (length definition, mean, range and SD): no data

- Valve height at study initiation, for shell deposition study (mean and range, SD): no data

- Food type: fed with unicellular green fresh-water algae *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*.

- Amount: no data

- Frequency: no data

Study design

Test type

static

Water media type

Freshwater

Limit test

yes

Total exposure duration

48 h

Test conditions

Test temperature

Continuously recorded in one test flask, remained between 18 and 22°C and was constant within $\pm 1^\circ\text{C}$.

Dissolved oxygen

Measured using a WTW OXI 538 oxymeter. Validity criteria: $>$ or $= 3$ mg/L in control and test vessels. measured data: at T0 and C0, Dissolved O2 = 8.4 mg/L at T0 and C= 100mg/L, Dissolved O2 = 8.5 mg/L at T48h and C0, Dissolved O2 = 8.1mg/L at T48h and C= 100mg/L, Dissolved O2 = 8.3mg/L.

Salinity

Fresh water test

Details on test conditions

TEST SYSTEM

- Test vessel: 30 mL glass bottles stoppered with PTFE bungs and sealed with aluminium caps. Approximately 90% of the final volume of the test suspensions was added into test flasks.

- Material, size, headspace, fill volume: In order to keep GRAPHISTRENGTH C100 in suspension, flasks were continuously maintained under axial rotation by use of a cylindrical roller device.

Physical-chemical parameters were measured using a METTLER TOLEDO 345 pH-meter for measurement of pH and with a WTW OXI 538 oxymeter for dissolved oxygen measurement. Each test flask was totally filled with the test suspension (30 mL).

- Aeration: until oxygen saturation.

- Type of flow-through (e.g. peristaltic or proportional diluter):

- Renewal rate of test solution (frequency/flow rate): no data

- No. of organisms per vessel: Five *Daphnia magna* were added into each test flask, for preliminary and definitive tests.

- No. of vessels per concentration (replicates): Concerning the preliminary test: Two replicates were prepared for each concentration.

Concerning the definitive test: Four replicates were prepared for each concentration.

- No. of vessels per control (replicates): Concerning the preliminary test: Two control flasks were prepared, with no test item.

Concerning the definitive test: Four control flasks were prepared with no test item.

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: ultra-pure water was used for the preparation of dilution water.

Osmotically filtered water was used to prepare the dilution water: Chemical reagents used for the preparation of dilution water are of « analytical grade ». Dilution water is prepared according to the protocol described in standard NF EN ISO 6341:

The following stock solutions are prepared: CaCl₂, 2H₂O: 11.76 g per litre of water, MERCK Ref. 1.02382.5000 batch A935182902 MgSO₄, 7H₂O: 4.93 g per litre of water, MERCK Ref. 1.05886.1000 batch A975086904 NaHCO₃: 2.59 g per litre of water, MERCK Ref. 1.06329.0500 batch

K39347429919KCI: 0.23 g of per litre of water, MERCK Ref. 1.04936.0250 batch K37429936851 Dilution water is obtained by adding 25 mL of each of the stock solutions in a final volume of 1 litre and then aerating the solution until oxygen saturation. Sum of Ca and Mg ions in dilution water is of 2.5 mmol/L and ratios of Ca/Mg et Na/K are 4 and 10.

- Total organic carbon:
- Particulate matter:
- Metals:
- Alkalinity:
- Ca/mg ratio:
- Conductivity:
- Culture medium different from test medium:
- Intervals of water quality measurement:

OTHER TEST CONDITIONS

- Adjustment of pH: no
- Photoperiod: no data
- Light intensity: Test flasks were incubated in darkness

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) : immobilisation

TEST CONCENTRATIONS

- Spacing factor for test concentrations:
- Justification for using less concentrations than requested by guideline:
- Range finding study: In the preliminary test, daphnids were exposed under static conditions to a series of 6 concentrations (100, 50, 10, 5, 1 and 0.1 mg/L) of GRAPHISTRENGTH C100 in dilution water.
- Test concentrations: Based on the results of preliminary test, it was decided to perform a limit test at 100 mg/L. Volumetric flasks were filled with the required volumes of the test item stock suspension.

Reference substance (positive control)

not required

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC50	> 100 mg/L	nominal	test mat.	mobility	

Details on results

No immobilization up to the highest tested concentration 100 mg/L.

Results with reference substance

- Results with reference substance valid? yes

The sensitivity of the test system and the methodology are evaluated every month by performing an inhibition test on potassium dichromate. The nearest value of EC50 obtained on 17/02/10 was 0.78 mg/L. For reference, ISO 6341 has a validity criteria specifying that EC50 must be in the range 0.6 to 2.1 mg/L.

Reported statistics and error estimates

no

Any other information on results incl. tables

Table 1: Dissolved O2 and pH measured at the beginning and at the end (48h) of the exposition.

Nominal concentration (mg/L)	pH		Dissolved O2 (mg/L)	
	T0	T48h	T0	T48h
0 (T)	7.95	7.77	8.4	8.1
100	7.95	7.73	8.5	8.3

Table 2: Range finding test-Percentage of Daphnia magna immobilisation

Nominal concentration (mg/L)	Immobilisation (%)	
	24h	48h
T	0	0
100	0	0
50	0	0
10	0	0
5	0	0
1	0	0
0.1	0	0

Table 3: Definitive test-Percentage of Daphnia magna immobilisation

Nominal concentration (mg/L)	immobilisation	
	24h	48h
T	0	0
100	0	0

Table 4: EC50 values determined at 24 and 48h post exposure based on nominal concentrations.

Endpoint	EC50 value (mg/L)	NOEC (mg/L)
24h	>100	ND
48h	>100	ND

Applicant's summary and conclusion

Conclusions

The determination of the inhibition of mobility of *Daphnia magna* exposed to the test item GRAPHISTRENGTH C100 for a duration of 48 hours was assessed according to the OECD Guideline 202. *Daphnia magna* were exposed under static conditions to GRAPHISTRENGTH C100 dispersed in dilution water. The concentration of test item causing a 50 % reduction in mobility (EC50) was estimated.

Executive summary

The acute toxicity of the test item GRAPHISTRENGTH C100, was evaluated with *Daphnia magna* in a study performed in accordance with OECD testing guideline 202 and GLP requirements.

The young daphnids were exposed to nominal test concentrations between 0.1 and 100 mg/L under static conditions during 48 hours. EC50-48 h was estimated using nominal concentrations as being greater than 100 mg/L.

As no immobilisation was observed up to 100 mg/L (the highest tested concentration) no EC50 could be determined. EC0 is greater or equal to 100 mg/L.

Mortality in control, temperatures, pH range and dissolved oxygen concentration were reported and in agreement with the guideline requirements.

Endpoint study record: Nanocyl NC7000 (TG 202) Short-term toxicity to aquatic invertebrates.002

Administrative Data

Purpose flag key 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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	

GLP compliance

no data

Test materials

Test material identity

Identifier	Identity
other:	Nanocyl NC7000

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC50	> 100 mg/L				

Endpoint study record: Nikkiso MWCNT (TG 202) Short-term toxicity to aquatic invertebrates.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 Following OECD TG

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			CERI	95398			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Nikkiso MWCNT

Vehicle

yes

Details on test solutions

Test substance: 10 mg/L (this level was determined based on a preliminary test), Dispersing agent (HCO-40): 100 mg/L

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

TEST ORGANISM

- Strain: daphnia magna
- Source: university of Sheffield

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

48 h

Remarks No aeration, No change of solution

Test conditions

Hardness

27 mg/L (as CaCO₃)

Test temperature

20+/-1 C

pH

7.3

Nominal and measured concentrations

Test substance: 10 mg/L

Details on test conditions

Test Item: immobility, abnormal behaviour or appearance
Number of species per sex per dose: Five daphnids in each test (n=4), control (n=4) or vehicle control (n=4) solution
Exposure duration: 48 hours, Light: 16 hours photoperiod daily, Temperature: 20 ± 1 °C, Feed: none

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC50	> 10 mg/L	nominal	test mat.	mobility	

Any other information on results incl. tables

Immobility

Nominal concentration (mg/L)		Immobility (%)			
		24 hours		48 hours	
		Replicate	Test level	Replicate	Test level
Control	A	0	0	0	0
	B	0		0	
	C	0		0	
	D	0		0	
Vehicle control	A	0	0	0	0
	B	0		0	
	C	0		0	
	D	0		0	
10	A	0	0	0	0
	B	0		0	
	C	0		0	
	D	0		0	

Observed abnormal response

Nominal concentration (mg/L)		Immobility (%)			
		24 hours		48 hours	
		Immobilization	Other symptom	Immobilization	Other symptom
Control		-	-	-	-
Vehicle control		-	-	-	-
10		-	-	-	-

6.1.4 Long-term toxicity to aquatic invertebrates

Endpoint study record: Graphistrength C100 (TG211) Long-term toxicity to aquatic invertebrates.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 Guideline, GLP compliant

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2010		REACH Dossier; Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					2010-10-19

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 211 (Daphnia magna Reproduction Test)	

GLP compliance

yes (incl. certificate)

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:	graphistrength C100

Analytical monitoring

no

Vehicle

no

Details on test solutions

Between 5 and 100 mg/L (suspensions were renewed every two or three days). Stirring of the suspension was ensured.

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

Biological reagent: organisms Daphnia magna Straus (Cladocera, Crustacea), clone 5 or clone A. Animals come from a stock breeding in the laboratory reared in VOLVIC® water added with vitamin B12, Na₂SeO₃, 5H₂O, spiruline, Calcium, Magnesium unicellular green fresh-water algae Pseudokirchneriella subcapitata and Chlorella vulgaris. Selection of test organisms less than 24 hours old is made by filtration.

Study design

Test type

semi-static

Water media type

Freshwater

Limit test

no

Total exposure duration

21 d

Test conditions***Test temperature***

19.4 ± 2.6 °C Variation in temperature was higher than expected, due to a temperature control issue in the laboratory

pH

Fresh solutions: 7.8 - 8.1

Dissolved oxygen

Fresh solutions: 8.9 - 9.3

Nominal and measured concentrations

Nominal concentrations: 0 - 5 - 10 - 22 - 47 - 100 mg/L

Details on test conditions

Dilution water was prepared with Volvic® water complemented as follow: 0.1 ml/L of B12 vitamin solution (1 µg/L) 0.1 ml/L of Na₂SeO₃·5H₂O solution (6.7 µg/L) 1ml/L of calcium / magnesium solution: Ca(NO₃)₂·4H₂O : 208 g/L and MgCl₂·6H₂O : 28 g/L. These solutions were prepared with ultra-pure water (resistivity > 18Mohm), reagents « analytical grade »

Reference substance (positive control)yes (K₂Cr₂O₇)**Results and discussions****Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	NOEC	47 mg/L	nominal	test mat.	reproduction	
21 d	EC50	317.75 mg/L	nominal	test mat.	reproduction	
21 d	LOEC	> 100 mg/L	nominal	test mat.	reproduction	

Any other information on results incl. tables

GRAPHISTRENGTH C100 Nominal Concentrations (mg/L)	0.00	5.00	10.00	22.00	47.00	100.00
Vessel 1	222	192	213	171	169	26
Vessel 2	193	222	192	190	109	170
Vessel 3	208	180	190	192	169	191
Vessel 4	202	207	201	183	181	158
Vessel 5	218	0	183	189	157	166
Vessel 6	158	174	198	194	170	173
Vessel 7	165	197	185	214	211	156
Vessel 8	183	203	192	156	158	161
Vessel 9	208	186	0	179	176	185
Vessel 10	180	218	184	196	197	143
Ne Total	1937	1779	1738	1864	1697	1529
Replicates number	10	9	9	10	10	10
Mean	193.70	197.67	193.11	186.40	169.70	152.90
Standard deviation	21.78	64.40	61.74	15.60	27.08	46.73
Coefficient of variation (%)	11.25	36.20	35.52	8.37	15.96	30.56
% Inhibition	-	-2.05	0.30	3.77	12.39	21.06

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

The chronic toxicity (inhibition of reproduction) of the test item GRAPHISTRENGTH C100 to Daphnia magna was assessed according to the guideline 211 of the OECD. Daphnia were exposed to a range of concentrations of GRAPHISTRENGTH C100 dispersed in dilution water. The chronic effect measured during the assay was the inhibition of reproduction after time periods of 21 days. EC50, LOEC and NOEC values are summarised in the following table: Measured concentrations EC50 Value LOEC NOEC mg/L 317.75 100 47

Endpoint study record: Nanocyl NC7000 (TG211) Long-term toxicity to aquatic invertebrates.002

Administrative Data

Purpose flag	key 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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 211 (Daphnia magna Reproduction Test)	

GLP compliance

no data

Test materials

Test material identity

Identifier	Identity
other:	Nanocyl NC7000

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
	LOEC	> 25 mg/L				

Endpoint study record: Nikkiso MWCNT (TG211) Long-term toxicity to aquatic invertebrates.001

Administrative Data

Purpose flag

key study

Study result type

experimental result **Study period** 2011

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies Following OECD TG

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			Mitsubishi Chemical Medience	A100702			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 211 (Daphnia magna Reproduction Test)	

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
	Nikkiso MWCNT

Vehicle

yes

Details on test solutions

Test substance: 0.0030, 0.0095, 0.030, 0.095, 0.30 mg/L, Dispersing agent (Tween 80): 3.0 mg/L
Control: Control (no test substance and dispersing agent) and Vehicle control (dispersing agent only)

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

TEST ORGANISM

- Strain/clone: Daphnia magna
- Source: national Institute for Environmental Studies

Study design

Test type

semi-static

Limit test

yes

Total exposure duration

21 d

Test conditions

Test temperature

20 +/- 1 C

Details on test conditions

Test Item: number of living offspring, mortality of the parent animals

Number of species per sex per dose: Ten daphnids in each test, control or vehicle control solution

No aeration, Change of solution: every 2 days

Exposure duration: 21 days, Light: 16 hours photoperiod daily, Temperature: 20+/-1 C

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	NOEC	0.3 mg/L	nominal	test mat.	reproduction	
21 d	LOEC	> 0.3 mg/L	nominal	test mat.	reproduction	
21 d	EC50	> 0.3 mg/L	nominal	test mat.	mortality	
21 d	LC50	> 0.3 mg/L	nominal	test mat.	reproduction	

Any other information on results incl. tables

Mean Cumulative Number of Offsprings Produced per Parent Alive ($\Sigma FI/P$)

Test Group	Nominal Conc. (mg/L)	$\Sigma FI/P$														
		Exposure time (days)														
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	--	0.0	13.8	16.5	21.9	21.9	53.8	53.8	81.0	88.4	88.4	88.4	106.8	109.5	114.0	137.4
Solve. control	--	0.0	15.7	15.7	19.4	19.4	55.8	55.8	94.0	94.0	94.0	94.0	117.3	117.3	120.0	153.0
1	0.0030	0.0	12.5	14.3	24.4	24.4	52.0	52.0	85.8	90.9	90.9	90.9	112.5	115.8	123.5	146.5
2	0.0095	0.0	16.2	16.2	27.7	27.7	52.8	52.9	90.9	91.2	91.2	91.2	117.6	117.6	125.7	152.0
3	0.030	0.0	17.7	17.7	32.7	32.7	53.1	53.1	91.7	91.8	91.8	91.8	119.9	119.9	132.7	155.0
4	0.095	0.0	15.1	15.1	19.0	19.0	55.4	55.6	96.7	96.8	96.8	96.8	121.8	121.8	128.4	158.1
5	0.30	0.0	14.0	15.2	28.0	28.0	54.4	54.4	91.4	93.4	93.4	93.4	123.3	123.3	130.1	154.1

Reproduction Rate, Inhibition Rate and Results of Statistical Comparison

Test Group	Nominal Conc. (mg/L)	Cumulative Number of Offsprings										Mean	S. D.	Reprod. Rate (%)	Inhibition Rate	Signific. diff.
		Vessel No.														
		1	2	3	4	5	6	7	8	9	10					
Control	--	14.6	15.3	14.3	14.9	15.5	15.0	10.9	15.2	15.6	61	137.4	30.1	--	--	--
Solve. control	--	15.0	15.0	16.3	16.9	15.2	14.1	14.8	14.9	15.3	15.5	153.0	7.9	--	--	--
1	0.0030	14.5	D	16.3	11.2	14.7	15.0	15.7	14.8	D	15.0	146.5	15.1	95.8	4.2	--
2	0.0095	15.0	15.0	15.5	14.6	14.8	15.9	14.7	14.5	16.8	D'	152.0	7.5	99.3	0.7	--
3	0.030	15.3	15.1	16.9	16.1	17.2	13.6	14.1	15.6	15.9	15.2	155.0	11.2	101.3	-1.3	--
4	0.095	14.5	14.7	D	15.0	17.3	15.5	16.0	17.2	15.7	16.4	158.1	10.2	103.3	-3.3	--
5	0.30	15.6	15.4	16.5	D'	16.2	12.4	16.7	14.8	15.6	15.5	154.1	12.7	100.7	-0.7	--

Overall remarks, attachments

Illustration (picture/graph)

Figure 1 Cumulative Mortality of Parent *Daphnia*

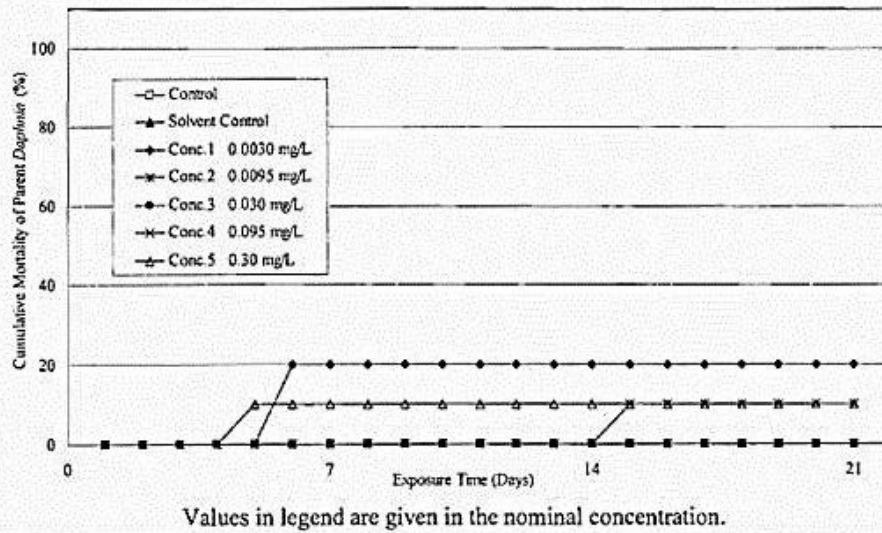
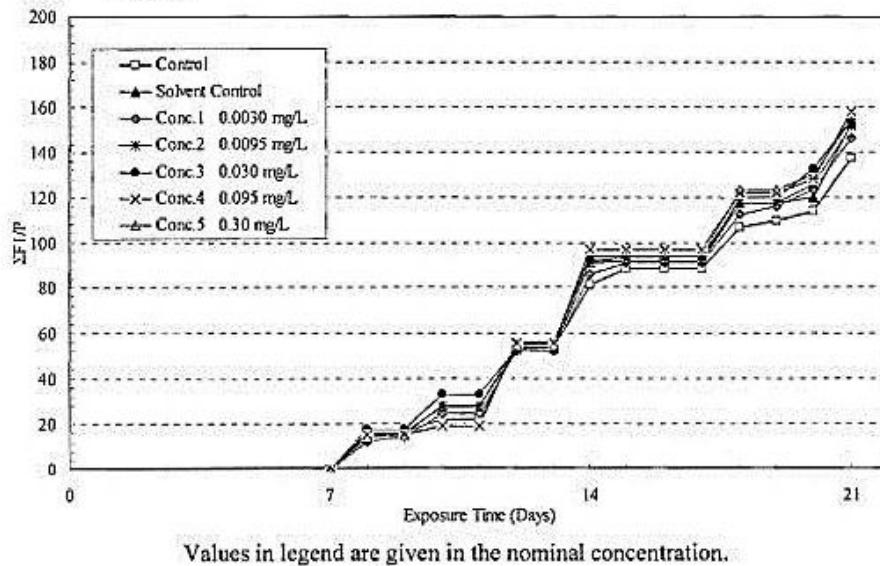


Figure 2 Mean Cumulative Numbers of Offsprings Produced per Parent *Daphnia* Alive ($\Sigma F1/P$)



6.1.5 Toxicity to aquatic algae and cyanobacteria

Endpoint study record: Baytube (TG201) Toxicity to aquatic algae and cyanobacteria.003

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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	

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

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
72 h	EC50	ca. 134 mg/L				

Endpoint study record: Graphistrength C100 (TG201) Toxicity to aquatic algae and cyanobacteria.002

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2010		REACH Dossier; Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					2010-04-20

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Arkema Graphistrength C100

Analytical monitoring

yes

Details on sampling

- Concentrations: .As data were available on a very similar test item, no preliminary test was performed. In the definitive test: 1000, 500, 230, 105, 48, 22 and 10 mg/L.
- Sampling method: For definitive test: suspensions of GRAPHISTRENGTH C100 were directly prepared for each concentration by weighing 10, 22, 48, 105, 230, 500 and 1000 mg into 100 mL of dilution water and adjusted to 1L. Then 50 mL of each suspension under stirring were transferred to the corresponding test flasks, where algae were added to reach 10⁴ cells per mL.

After 24, 48 and 72h of incubation, about 1 mL was sampled from each test flask under. Inoculated control flask (T) and non-inoculated blank (BI) were prepared and incubated.

- Sample storage conditions before analysis: samples were stored in darkness until determination of algae concentration.

Vehicle

yes

Details on test solutions

PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances)

- Method: The stock solution will be prepared by dispersing the test item in reconstituted water made up by diluting pure mineral salts (analytical grade) in ultra-pure water. The test solutions will then be prepared by diluting the stock solution in reconstituted water under stirring. The test solutions will be placed into glass vessels together with algae from a pre-culture.

- Controls: For preliminary test: an inoculated control flask (T) was prepared and incubated with no test item. A non-inoculated blank (BI) containing only dilution water without test item was also prepared and incubated. For definitive test: an inoculated control flask (T) was prepared and incubated with no test item. A non-inoculated blank (BI) containing only dilution water without test item was also prepared and incubated. Test flasks and blanks were prepared in triplicate. Six replicates were used for the control.

- Chemical name of vehicle (organic solvent, emulsifier or dispersant): dilution water

Test organisms

Test organisms (species)

Pseudokirchnerella subcapitata

Details on test organisms

- Strain: *Pseudokirchnerella subcapitata*, CCAP 278/4 stock

- Source (laboratory, culture collection): the Culture centre of Algae and Protozoa

- Age of inoculum (at test initiation): no data

- Method of cultivation: described in Annex 2 of the OECD 201 guideline.

Two flasks, each containing approximately 100 mL of axenic stock culture of algae are incubated at 23±1°C under lighting, slowly continuously shaken. These stock cultures are renewed every week, using two new cultures. The quality of the stock culture was verified for the absence of micro-organisms and deformed cells under microscopic observation before use. Three days before the beginning of the study, two pre-cultures were prepared by inoculating each stock suspension of algae (5 mL) into sterile dilution water (500 mL). The pre-cultures were incubated under the same conditions as those used for the stock cultures. Only one of the two pre-cultures was used to inoculate the test flasks. The second one was to be used only if the first one was damaged.

Study design

Test type

static

Water media type

freshwater

Limit test

no

Test conditions

Test temperature

23+/-1°C

Details on test conditions

TEST SYSTEM

- Test vessel: 120 mL glass bottles stoppered with cellulose bungs.
- Type (delete if not applicable): closed
- Material, size, headspace, fill volume: The incubation was performed in a phytoculture cabinet. Flasks carrying trays are continuously rotating at 20 rpm and constantly illuminated. Filling volume: 50 mL. Determination of algae concentration was carried out using cytofluorimetry with a Cytofluor 2350 device.
- Aeration: no data
- Type of flow-through (e.g. peristaltic or proportional diluter): no data
- Renewal rate of test solution (frequency/flow rate): every week
- Initial cells density: 10^4 cells/mL
- No. of vessels per concentration (replicates): concerning the definitive test, test flasks and blanks were prepared in triplicate.
- No. of vessels per control (replicates): 6 replicates were used for the control.

GROWTH MEDIUM

- Standard medium used: no data

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: according to the protocol described in the Annex 3 of the OECD Guideline 201.

OTHER TEST CONDITIONS

- Sterile test conditions: no data
- Adjustment of pH: no data
- Photoperiod: 16 hours of illumination, 8 hours of darkness
- Light intensity and quality: flasks are illuminated by 8 fluorescent tubes between 6,000 and 10,000 lux.

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) :

- Determination of cell concentrations: [counting chamber; electronic particle counter; fluorimeter; spectrophotometer; colorimeter] 3 replicates test chambers in each treatment, Cytofluorimeter with a Cytofluor 2350 device.

Reference substance (positive control)

yes (Potassium dichromate)

Any other information on materials and methods incl. tables

Toxicity to green algae (*Pseudokirchneriella subcapitata*) was examined by using TG 201 (under GLP conditions) with nominal concentrations between 10 and 1000 mg/L.

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
72 h	EC50	120 mg/L	nominal	act. ingr.	growth rate	
72 h	NOEC	< 10 mg/L	nominal	act. ingr.	biomass	

Details on results

The biomass in the control cultures has increased exponentially by a factor of 163 higher than 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day⁻¹. See Tables 1 and 2. It was observed that most of GRAPHISTRENGTH C100 particles did not remain as suspension and gathered together in the lower part of each flask. Microscopic observation confirmed that the algae appeared normal at the end of the test. An increase in the pH is observed. This may be associated to the consumption of the dissolved CO₂ due to the growth of algae.

Any other information on results incl. tables

For the definitive test: table 1

Nominal concentration (mg/L)	Average cell density (cell/mL)				Average Biomass	Average growth rates
	T0	T24h	T48h	T72h		
0 (T)	1.00E+04	8.55E+04	3.20E+05	1.63E+06	2.87E+07	1.698
1000	1.00E+04	1.70E+04	2.22E+03	0.00E+00	0.00E+00	0.000
500	1.00E+04	3.44E+04	1.18E+04	1.18E+04	6.52 E+05	0.056
230	1.00E+04	3.29E+04	9.99E+03	2.96E+04	7.85E+05	0.362
105	1.00E+04	3.88E+04	4.59E+04	2.60E+05	4.55E+06	1.086
48	1.00E+04	4.29E+04	1.12E+05	6.49E+05	1.09E+07	1.391
22	1.00E+04	7.84E+04	2.61E+05	1.38E+06	2.41E+07	1.643
10	1.00E+04	7.69E+04	2.37E+05	1.40E+06	2.37E+07	1.647

Table 2

Nominal concentration (mg/L)	Average % inhibition of cell growth (%)	Average % inhibition of growth rate (%)
1000	100	100
500	98	97
230	97	79
105	84	36
48	62	18
22	16	3
10	17	3

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

- The biomass in the control cultures has increased exponentially by a factor of 163 higher than 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day⁻¹.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures did not exceed 35%. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 7%.

Executive summary

The determination of the growth inhibition of the freshwater algae *Pseudokirchneriella subcapitata* (previously known as *Raphidocelis subcapitata* and *Selenastrum capricornutum*) exposed to the test item GRAPHISTRENGTH C100 for a duration of 72 hours was assessed according to the OECD Guideline 201.

Algae were exposed to 10 to 1000 mg/L of GRAPHISTRENGTH C100 dispersed in dilution water. The toxic effect measured during the assay was the inhibition of cellular multiplication over a time period of 72 hours.

The concentrations of test item causing a 50 % reduction in biomass (E_bC_{50}) and in growth rate (E_rC_{50}) were estimated. It was possible to determine No Observable Effect Concentrations (NOEC).

E_bC_{50} -72h, based on biomass, was of 38 mg/L and E_rC_{50} -72h, based on growth rate, was of 120 mg/L. NOEC, based on biomass, was <10 mg/L and NOEC, based on growth rate, was of 22 mg/L.

Endpoint study record: Graphistrength C100 Toxicity to microorganisms.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	GLP 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		2010		REACH Dossier; Dossier ID: DISS-abda73cd-a2cd-3801-e044-00144f67d249					2010-05-08

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	

GLP compliance

yes (incl. certificate)

Test materials

Test material identity

Identifier	Identity
	Graphistrength C100

Analytical monitoring

yes

Details on sampling

- Concentrations:

Limit test: As precedent toxicity experiments showed very low effect, it was decided to test GRAPHISTRENGTH C 100 at final concentrations of 500 mg/L and 5000 mg/L. Each suspension was prepared separately by weighing of the convenient amount of the test item in 16 mL of the synthetic sewage feed and dilution to the final concentration.

Implementation of tests:

The tests were carried out in 500 ml flasks in the following repartition: 16 mL of the synthetic sewage feed containing the test item, adequate volume of ultra-pure water, 20 mL of sludge suspension. At T0, the first control (control 1) without test item is prepared. Then, every 15 min, the other vessels with different concentrations of item are prepared in turn. Finally the second control (control 2) is prepared. All suspensions are aerated and stirred during 3 hours and incubated at 20+/-2°C without light. Then a sample of each mixture is poured into a BOD bottle. The oxygen electrode is inserted into the bottle. The oxygen concentration is measured every minute in a period of 10 min. The dissolved oxygen concentrations are determined electrochemically using an oxygen electrode and meter.

- Sampling method: Test solution of 3-5 dichlorophenol: A 0.5 g/L stock solution of 3-5 dichlorophenol in ultra-pure water is used for preparing the different tested solutions from 2.5 to 40 mg/L with factor of 2.5.

- Composition of synthetic sewage feed: A synthetic sewage feed is made by dissolving the following solutions in 1 L of ultra-pure water: 16 g peptone, 11 g meat extract, 3 g urea, 0.7 g sodium chlorure, 0.4 g calcium chlorid dihydrate $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.25 g magnesium sulfate heptahydrate $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.8 g dipotassium hydrogen phosphate K_2HPO_4 .

Vehicle

yes

Test organisms

Test organisms (species)

activated sludge, domestic

Details on inoculum

Preparation of inoculum: The inoculum comes from the activated sludge of the biological domestic wastewater treatment plant from the city of Abidos.

The effluent, obtained one day before seeding the flasks, is centrifuged at approximately 20 °C for 20 min at 4000g.

The base is re-suspended in ultra-pure water in order to keep the concentrations unchanged with respect to the sample.

The amount of dry material in the inoculum is determined by desiccation at 105°C until a constant weight. From this result the amount of wet sludge can be calculated in order to obtain an activated sludge with a mixture liquor suspended solids level of 4 g/L. The inoculum is preconditioned (aeration). Every day, 50 ml/L of synthetic sewage feed are added until the day the flasks are inoculated. Values of suspended solids were 4.6 g/L. Then a dilution was used to obtain 1 g/L. This level corresponds to a dose of 0.4 g/L in the test medium.

Study design

Test type

other: stirring by means of a covered magnet

Water media type

freshwater

Limit test

yes

Total exposure duration

3 h

Post exposure observation period

after the 3 h of exposure

Test conditions

Test temperature

20±2°C

Nominal and measured concentrations

2.5, 6.4, 16 and 40 mg/L for the principal tests
500 and 5000 mg/L for the limit test

Reference substance (positive control)

yes (3,5 dichlorophenol)

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
3 h	EC50	> 5000 mg/L	nominal	test mat.	respiration rate	

Results with reference substance (positive control)

The sensitivity of the test system and the methodology is evaluated with 3,5 dichlorophenol. An EC50-3h

value of 14 mg/L was obtained, which is within the validity specified range from 5 to 30 mg/L.

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Quality criteria of the study:

- The difference in respiration rates between the two controls was 5% which is below 15%- the EC50 of 3,5 dichlorophenol is 14 mg/L which is between 5 and 30 mg/L

Executive summary

The purpose of the study was to determine the effect of the test item on bacteria according to the OECD guideline 209 "Activated sludge, respiration inhibition test"

The effect is assessed by measuring the microorganisms respiration rate on a sample of activated sludge during an incubation period of 3 hours.

The method was applied with respect to the different criteria:

- The difference of respiration rates between the two controls was below 15%

- EC50 of the control test with the reference item 3,5 dichlorophenol was between 5 and 30 mg/L (14 mg/L)

Two nominal concentrations were tested: 500 and 5000 mg/L. GRAPHISTRENGTH C 100 did not show any effect on the respiration of the activated sludge in the conditions of the test at the concentration of 500 mg/L. At 5000 mg/L an inhibition of respiration of 42.6 % was observed. In conclusion, EC5 0-3h is higher than 5000 mg/L

Endpoint study record: Nanocyl NC7000 (TG201) Toxicity to aquatic algae and cyanobacteria.001

Administrative Data

Purpose flag key 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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	

Test materials

Test material identity

Identifier	Identity
other:	Nanocyl NC7000

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
	EC50	ca. 8.4 mg/L				

Endpoint study record: Nikkiso MWCNT T(TG201) oxicity to aquatic algae and cyanobacteria.001

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			CERI	95397			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
other:	Nikkso MWCNT

Details on test solutions

Test substance concentration: 0.10, 0.32, 1.0, 3.3, 10 mg/L

Dispersing agent 100 mg/L

Control: Control (Culture media) and Vehicle control (dispersing agent only)

Test organisms

Test organisms (species)

Pseudokirchnerella subcapitata

Study design

Test type

static

Total exposure duration

72 h

Test conditions

Any other information on materials and methods incl. tables

Test organism: *Pseudokirchnerella subcapitata*

Initial biomass concentration: 0.75×10^4 cells/mL

Dispersing agent: HCO-40 (ethylene oxide adduct of hydrogenated castor oil)

Exposure duration: 72 hours

Light intensity: 90 mE/m²/s (setting value),

Temperature: 21 - 24°C

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
72 h	EC50	> 10 mg/L	nominal	test mat.	growth rate	
72 h	NOEC	0.32 mg/L	nominal	test mat.	growth rate	

Any other information on results incl. tables

Value of biomass at each time

Nominal Concentration (mg/L)	No.	Chlorophyll fluorescence value (relative unit)			
		0 hour	24 hours	48 hours	72 hours
Control	A	2.0	9.3	50	180
	B	2.0	10	50	190
	C	2.0	10	51	190
	Mean	2.0	10	51	190
	SD	0	0.67	0.68	5.1
Vehicle control	A	2.0	11	58	210
	B	2.0	11	64	230
	C	2.0	11	60	240
	D	2.0	9.9	53	210
	E	2.0	12	64	220
	F	2.0	10	58	200
	Mean	2.0	11	60	220
	SD	0	0.82	4.4	15
0.10	A	2.0	9.5	54	210
	B	2.0	11	53	210
	C	2.0	11	60	190
	Mean	2.0	11	5.6	210
	SD	0	0.87	3.7	8.9
0.32	A	2.0	9.9	52	180
	B	2.0	11	52	210
	C	2.0	11	54	200
	Mean	2.0	11	53	200
	SD	0	1.2	1.3	18
1.0	A	2.0	9.1	45	150
	B	2.0	11	49	190
	C	2.0	11	49	160
	Mean	2.0	10	48	170
	SD	0	0.96	2.6	22
3.2	A	2.0	11	44	130
	B	2.0	11	42	140
	C	2.0	9.9	40	130
	Mean	2.0	10	42	140
	SD	0	0.37	1.7	4.2
10	A	2.0	6.4	20	47
	B	2.0	7.2	19	52
	C	2.0	7.3	18	49
	Mean	2.0	7.0	19	49
	SD	0	0.50	0.32	2.6

Growth rate and growth inhibition

Nominal Concentration (mg/L)	No.	Growth rate (0-3d)	Inhibition rate (%)
Control	A	1.50	3.7
	B	1.52	2.6
	C	1.51	3.4
	Mean	1.51	3.2
	SD	0.00910	0.58
Vehicle control	A	1.54	
	B	1.58	
	C	1.60	
	D	1.55	
	E	1.56	
	F	1.54	
	Mean SD	1.56 0.0230	
0.10	A	1.55	0.82
	B	1.55	0.78
	C	1.52	2.4
	Mean	1.54	1.3
	SD	0.0416	0.93
0.32	A	1.50	4.1
	B	1.56	0.41
	C	1.54	1.5
	Mean	1.53	2.0
	SD	0.0299	1.9
1.0	A	1.44	8.1
	B	1.52	2.7
	C	1.47	6.1
	Mean	1.47	5.6
	SD	0.0430	2.8
3.2	A	1.40	11
	B	1.42	9.3
	C	1.40	10
	Mean	1.41	10
	SD	0.0101	0.65
10	A	1.05	33
	B	1.08	31
	C	1.06	32
	Mean	1.06	32
	SD	0.0179	1.1

Table Result of statistical analysis

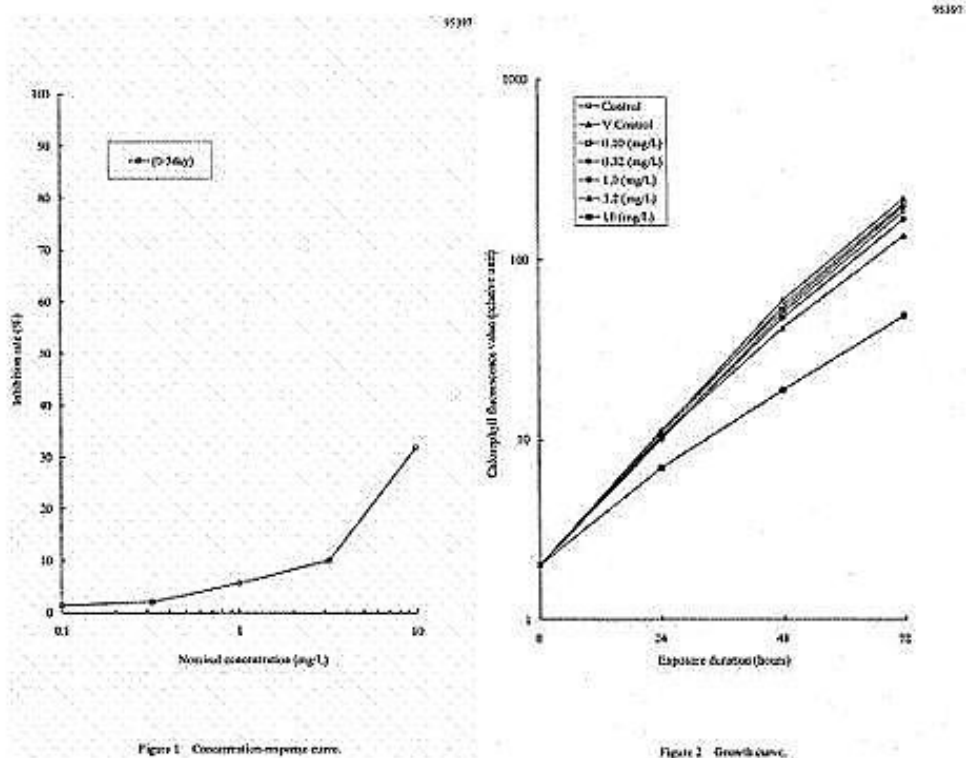
Nominal Concentration (mg/L)	Statistical analysis	Statistical procedure
0.10	n.s.	Bartlett's test One-way ANOVA Dunnett's multiple comparison test
0.32	n.s.	
1.0	**	
3.2	**	
10	**	

n.s.: No significant difference

***.: Significant difference (p<0.01)*

Overall remarks, attachments

Illustration (picture/graph)



6.1.6 Toxicity to aquatic plants other than algae

6.1.7 Toxicity to microorganisms

Endpoint study record: Baytubes (TG209) Toxicity to microorganisms.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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	

Test materials

Test material identity

Identifier	Identity
	Baytubes

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
3 h	EC50	> 10000 mg/L				

Endpoint study record: Nikkiso MWCNT (TG209) Toxicity to microorganisms.001

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			CERI	95936			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	

GLP compliance

yes

Test materials

Test material identity

Identifier	Identity
	Nikkiso MWCNT

Test organisms

Test organisms (species)

activated sludge of a predominantly domestic sewage

Study design

Limit test

yes

Total exposure duration

3 h

Test conditions

Test temperature

20 +/- 2 C

pH

7.5

Any other information on materials and methods incl. tables

Species: Activated sludge of a sewerage treatment plant (Kurume City, Japan)

Test substance; 100mg/L,

Reference substance (3,5 -Dichlorophenol); 4, 8, 16 and 32 mg/L

Test item; Oxygen uptake rate calculated from dissolved oxygen concentration

Study design

Activated sludge; 1,390 mg/L

Temperature; 20 +/- 2°C, Exposure Period; 3 h

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
3 h	EC50	> 100 mg/L	nominal	test mat.	respiration rate	

Details on results

EC50 > 100 mg/L

Results with reference substance (positive control)

EC50 = 6.75 mg/L

Any other information on results incl. tables

Oxygen consumption rate and respiratory inhibition rate

Test solution	Concentration (mg/L)	O2 consumption rate (mg O2 /L h)	Respiratory inhibition rate
Test material	100	36.6	0 (*)
Control (3,5 dichlorophenol)	4.0	21.1	33
	8.0	11.9	62
	16.0	9.61	69
	32.0	7.07	77
Control (blank)		30.7	
		31.8	

(*): As the calculation value became negative, the value set as zero.

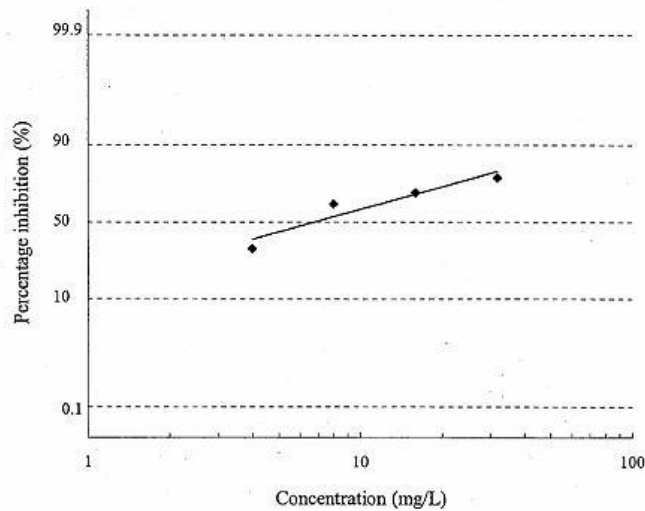
This result indicates that the test substance does not inhibit respiration of microorganism in activated sludge at 100 mg/L

Overall remarks, attachments

Illustration (picture/graph)

Study No. 95396

3-hour EC₅₀ = 6.75 mg/L (Probit)



Concentration (mg/L)	Percentage inhibition (%)
	3 hours
4.00	33
8.00	62
16.0	69
32.0	77

Fig. 1 Concentration - respiration inhibition curve (reference item).

6.2 Sediment toxicity

Endpoint study record: Graphistrength C100 (TG 219) Sediment toxicity.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 3 (not reliable)
Rationale for reliability incl. deficiencies Following OECD TG

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									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 219 (Sediment-Water Chironomid Toxicity Test Using Spiked Water)	yes (7 days observation)

Test materials

Test material identity

Identifier	Identity
other:	Graphistrength C100

Study design

Total exposure duration

7 d

Test conditions

Details on test conditions

Concentrations tested were from 0.1 to 50 mg/l. Mortality was observed.

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
7 d	LC50	6.15 mg/L				

Details on results

no chronic effect for surviving larvae.

6.3 Terrestrial toxicity

6.3.1 Toxicity to soil macroorganisms except arthropods

6.3.2 Toxicity to terrestrial arthropods

6.3.3 Toxicity to terrestrial plants

6.3.4 Toxicity to soil microorganisms

Endpoint study record: Toxicity to soil microorganisms.001

Administrative Data

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

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	AIST	2011			Mitsubishi Chemical Medience	A100703			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 216 (Soil Microorganisms: Nitrogen Transformation Test)	

GLP compliance

yes

Test materials**Test material identity**

Identifier	Identity
	Nikkiso MWCNT

Test organisms**Test organisms (inoculum)**

soil

Study design**Total exposure duration**

28 d

Test conditions***Test temperature***

20 +/- 2 C

Reference substance (positive control)

yes (C)

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
28 d	EC50	> 1000 mg/kg soil dw	nominal	test mat.	nitrate formation rate	

Overall remarks, attachments

Remarks on results including tables and figures

EC50 of the test material is more than 1000 mg / kg-dry sol

ED50 of the reference material (nitrapyrin) was 1.5 mg / kg-dry sol

Generation rate and inhibition rate

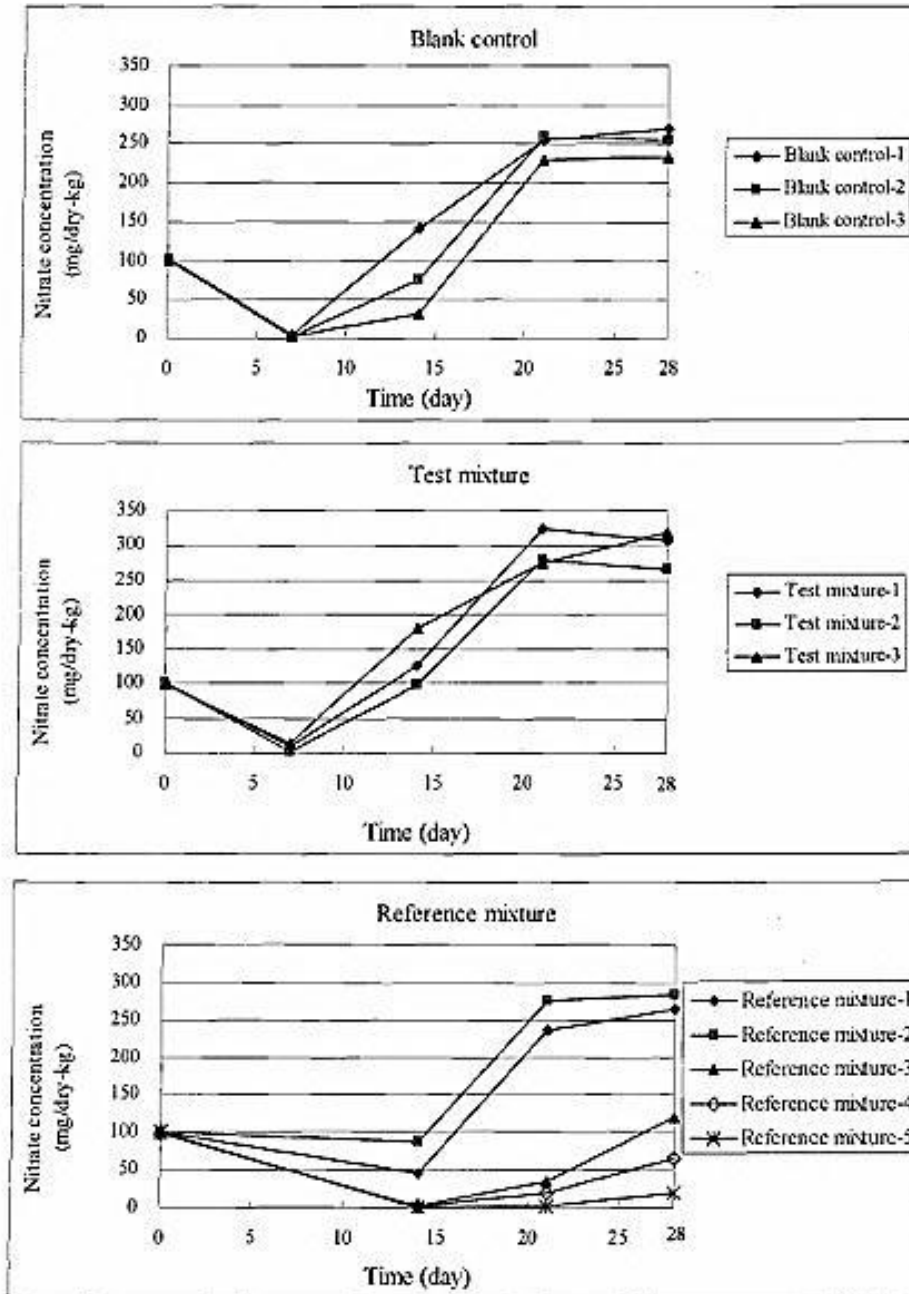
		0 day	7 day			14 day		
		Nitrate conc.	Nitrate conc.	Generat. rate	Inhibition rate	Nitrate conc.	Generat. rate	Inhibition rate
		mg/dry-kg	mg/dry-kg	%	%	mg/dry-kg	%	%
Blank control	1	103.7	4.2	-	-	142.0	-	-
	2	101.1	2.6	-	-	76.9	-	-
	3	100.5	2.8	-	-	30.8	-	-
	avg	101.8	3.2	-	-	83.2	-	-
Test mixture	1	101.6	8.3	259	0 (-159)	125.8	151	0 (-51)
	2	101.0	2.4	75	25	98.4	118	0 (-18)
	3	99.4	13.7	428	0 (-328)	181.4	218	0 (-118)
	avg	100.7	8.1	253	0 (-153)	135.2	163	0 (-63)
Ref. material	1	100.8	-	-	-	46.6	56	44
	2	100.5	-	-	-	86.8	104	0 (-4)
	3	98.9	-	-	-	3.1	4	96
	4	98.0	-	-	-	2.0	2	98
	5	99.6	-	-	-	<1.0	<2	>98

		21 day			28 day		
		Nitrate conc.	Generat. rate	Inhibition rate	Nitrate conc.	Generat. rate	Inhibition rate
		mg/dry-kg	%	%	mg/dry-kg	%	%
Blank control	1	254.2	-	-	270.0	-	-
	2	259.4	-	-	254.6	-	-
	3	230.0	-	-	234.9	-	-
	avg	247.9	-	-	253.2	-	-
Test mixture	1	323.2	130	0 (-30)	308.6	122	0 (-22)
	2	279.6	113	0 (-13)	264.4	104	0 (-4)
	3	274.3	111	0 (-11)	318.3	126	0 (-26)
	avg	292.4	118	0 (-18)	297.1	117	0 (-17)
Ref. material	1	236.2	-95	5	265.2	105	0 (-5)
	2	275.5	-111	0 (-11)	284.6	112	0 (-12)
	3	34.8	-14	86	120.1	47	53
	4	20.0	-8	92	66.6	26	74
	5	2.3	-1	99	18.9	7	93

Nitrate formation rate

		Nitrate formation rate (mg / dry-kg / day)		
		7-14 day	14-21 day	21-28-day
Blank control	1	19.7	16.0	2.3
	2	10.6	26.1	-0.7
	3	4.0	28.5	0.7
	average	11.4	23.5	0.8
Test mixture	1	16.8	28.2	-2.1
	2	13.7	25.9	-2.2
	3	24.0	13.3	6.3
	average	18.2	22.5	0.7
Reference mixture	1		27.1	4.1
	2		27.0	1.3
	3		4.5	12.2
	4		2.6	6.7
	5		0.3	2.4

Figure 1 Graph of nitrate concentration



6.4 Biological effects monitoring

6.5 Biotransformation and kinetics

6.6 Additional ecotoxicological information

Endpoint study record: Graphistrength C100 Xenopus laevi micronucleus assay (ISO21427-1)

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. deficiencies Following ISO

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: Infoemation from Arkema									

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: ISO 21427-1 Xenopus laevi micronucleus assay	

GLP compliance

no data

Test materials

Test material identity

Identifier	Identity
other:	Graphistrength C100

Results and discussions

Any other information on results incl. tables

No genotoxic effect