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

**DOSSIER ON SINGLE-WALLED CARBON NANOTUBES (SWCNT)
- PART 1 -**

**Series on the Safety of Manufactured Nanomaterials
No. 50**

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ENV/JM/MONO(2015)13/PART1
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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 50

**DOSSIER ON SINGLE-WALLED CARBON NANOTUBES (SWCNT)
- PART 1 -**



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**

Dossiers also published in the Series on the Safety of Manufactured Nanomaterials:

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No. 45, *Dossier on Cerium oxide (2015)*

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

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

No. 49, *Dossier on Multiwalled Carbon Nanotubes (MWCNTs) (2015)*

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

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

No. 53, *Dossier on Silver nanoparticles (2015)*

No. 54, *Dossier on Titanium dioxide (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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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; v) Mammalian Toxicology; and vi) Material Safety. They have been analysed using OECD Guidelines for the Testing of Chemicals (TG)⁸. The data is presented in an IUCLID⁹ style format and includes the protocols and methods used (see Preamble).

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

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

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

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

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

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

ACKNOWLEDGMENTS

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

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PART 2

7. TOXICOLOGICAL INFORMATION	ENV/JM/MONO(2015)13/PART2
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SUBSTANCE: SWCNT

1. GENERAL INFORMATION

1.1 Identification

Substance identification

Chemical name SWCNT

Public name Single-Walled Carbon Nanotubes

Type of substance

Composition mono constituent substance

Origin inorganic

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	Nikkiso SWCNT (Principal 1)		
	Trade name	Super Growth SWCNT (principal 2)		
	Trade name	Helix SWCNT (Alternate 1)		
	Trade name	CarboLex SWCNT (Alternate 2)		
	Trade name	CNI SWCNT (Alternate 3)		
	Trade name	Cheap Tubes SWCNT (Alternate 4)		
	Trade name	NIST SWCNT (Alternate 5)		
	Trade name	SWeNT SWCNT (Alternate 6)		
	Trade name	NASA SWCNT (Alternate 7)		
	Trade name	NanoIntegris SWCNT (Alternate 8)		
	Trade name	Carbon Solutions (Alternate 9)		

1.2 Composition

Substance composition

Name	Nikkiso SWCNT
Brief description	Principal SWCNT 1
Degree of purity	
>= 95 % (w/w)	
Impurities	
Typical concentration	>= 43700 ppm
Remarks	Fe
Typical concentration	>= 56 ppm
Remarks	Rb
Typical concentration	>= 22 ppm
Remarks	Zn
Typical concentration	>= 12 ppm
Remarks	Ga
Typical concentration	>= 10 ppm
Remarks	Cu
Typical concentration	>= 9 ppm
Remarks	Ni

Typical concentration ≥ 6	
Remarks	Pb

Name	Super Growth SWCNT
Brief description	Principal 2
Impurities	

Typical concentration ≥ 145 ppm	
Remarks	Fe

Typical concentration ≥ 103 ppm	
Remarks	Ni

Typical concentration ≥ 34 ppm	
Remarks	Cr

Typical concentration ≥ 11.5 ppm	
Remarks	Al

Typical concentration ≥ 2 ppm	
Remarks	Mn

Name	Helix SWCNT
Brief description	Alternate 1
Degree of purity	
ca. 90 % (w/w)	
Impurities	

Typical concentration $\geq 2.94 \%$ (w/w) Remarks Co	
Typical concentration $\geq 0.17 \%$ (w/w) Remarks Fe	
Typical concentration $\geq 1.213 \%$ (w/w) Remarks Mo	
Typical concentration $\geq 0.012 \%$ (w/w) Remarks Ni	
Typical concentration $\geq 0.024 \%$ (w/w) Remarks Si	
Typical concentration $\geq 0.01 \%$ (w/w) Remarks Ti	
Name Carbolex SWCNT Brief description Alternate 2 Impurities 	
Typical concentration $\geq 18.91 \%$ (w/w) Remarks Ni	
Typical concentration $\geq 4.511 \%$ (w/w)	

Remarks	Y
Typical concentration	$\geq 0.1 \%$ (w/w)
Remarks	Zn
Typical concentration	$\geq 0.174 \%$ (w/w)
Remarks	Si
Typical concentration	$\geq 0.055 \%$ (w/w)
Remarks	Fe
Typical concentration	$\geq 0.022 \%$ (w/w)
Remarks	Al
Typical concentration	$\geq 0.013 \%$ (w/w)
Remarks	Cu
Name	CNI SWCNT
Brief description	Alternate 3
Impurities	
Typical concentration	$\geq 8.371 \%$ (w/w)
Remarks	Fe
Typical concentration	$\geq 0.011 \%$ (w/w)
Remarks	Ni
Name	SWeNT SWCNT

Brief description	Alternate 6
Degree of purity	
>= 85— <= 90 % (w/w)	

1.3 Identifiers

1.4 Analytical information

1.5 Joint submission

1.6 Sponsors

Sponsors

Name	United States(Co-Lead Sponsor)
Type	member state
Contact persons	
Organisation	U.S. Environmental Protection Agency
Department	Chemical Safety for Sustainability (CSS),Office of Research and Development / IOAA
Title	Acting Deputy National Program Director
First name	Philip
Last name	Sayre
Phone	Office: 202 564 7673
Mobile	Mobile: 202 320 4603
E-mail	sayre.phil@epamail.epa.gov
Address	Ronald Regan Building and International Trade Center, Room 71177 1300 Pennsylvania Ave, NW Washington, D.C. 20004

Postal code	8101R
Country	United States

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	Principal SWCNT 1; Nikkiso SWCNT; e-DIPS (enhanced Direct-Injection-Pyrolytic-Synthesis) method is adopted for continuous production of SWCNTs. Principal SWCNTs 2; Super Growth; Water-assisted CVD using Fe catalyst developed by AIST (National Institute of Advanced Industrial Science and Technology) Alternate SWCNT 1; Helix; chemical vapor deposition Alternate SWCNT 2; Carbolex; carbon arc synthesis Alternate SWCNT 3; CNI; high-pressure CO disproportionation process (HiPCO) using Fe(CO) ₅ as a catalyst and purified by acid treatment
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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: Nikkiso SWCNT; Appearance/physical state/colour

Administrative Data

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

Data source**Reference**

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

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	other guideline: WPMN guidance manual	

Principles of method if other than guideline

TEM observation

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 SWCNT

Results and discussion**Colour**

black

Odour

odourless

Substance type

inorganic

Overall remarks, attachments

Remarks on results including tables and figures

The material is in sheet form consisting of entangled SWCNTs solid fibres (with a diameter of approximately 2nm) bundles with diameters of several decade nanometer.

Endpoint study record: Super Growth SWCNT; Appearance/physical state/colour

Administrative Data

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies Actual observation by AIST

Data source

Reference

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

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: WPMN guidance manual	

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:	Super Growth SWCNT

Results and discussion**Colour**

black

Odour

odourless

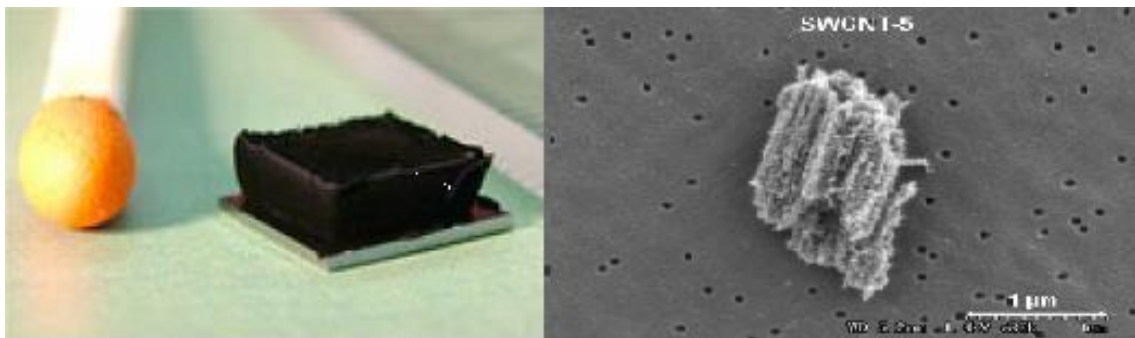
Substance type

inorganic

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

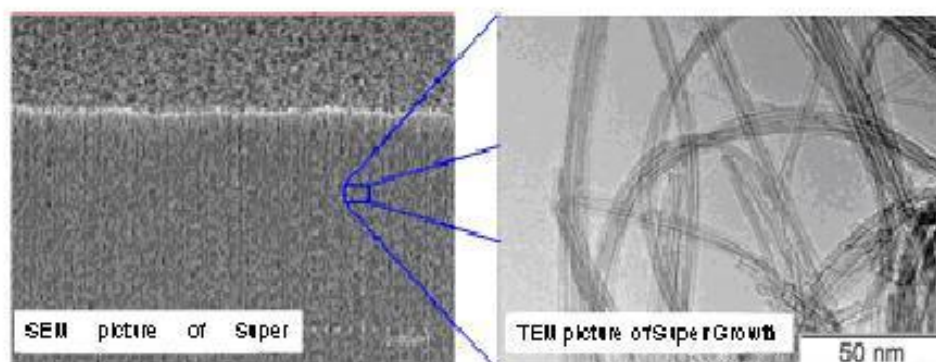
The material is grown in one direction on the substrate as shown in the picture below.
For the mammalian toxicity test, the test sample was well dispersed into the individual tube

Illustration (picture/graph)



Super Growth SWCNT; Comparing with match head (left) SEM picture (right)

SEM and TEM picture by magnification (under)



4.2 Melting point/freezing point

4.3 Boiling point

4.4 Density

4.5 Particle size distribution (Granulometry)

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

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Measured by 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 Manufactured Nanomaterials- Carbon Nanotubes						

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

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:	Nikkiso SWCNT

Test material form

dispersion

Details on test material

Dispersed SWCNT solution for the intratracheal instillation study

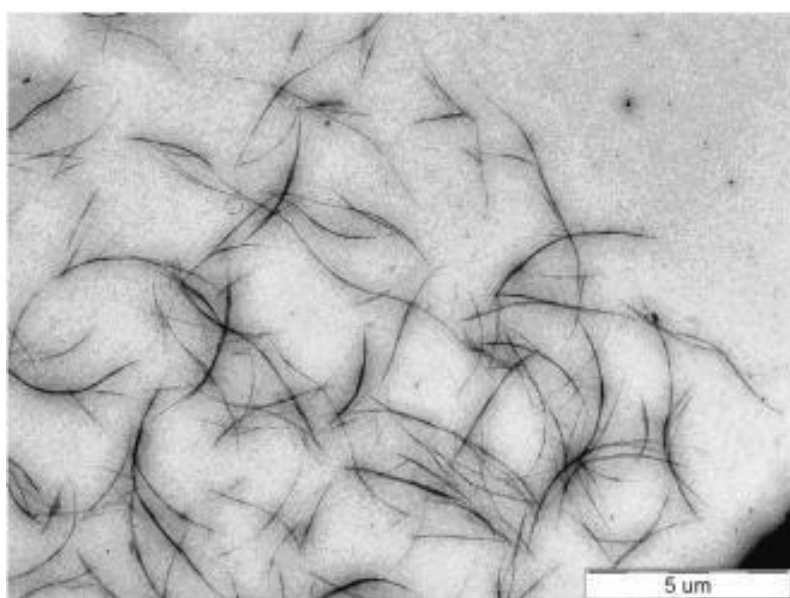
Results and discussions

Any other information on results incl. tables

width = 43.6 nm (SD = 1.6)
Length = 0.69 μm (SD = 2.1)

Overall remarks, attachments

Illustration (picture/graph)



Applicant's summary and conclusion

Executive summary

Measurement of samples for toxicity test by DLS; Diameter=2.7 μm (Standard Deviation=1.4)

Endpoint study record: Super Growth SWCNT; Particle size distribution (Granulometry).001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Measured by 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 Manufactured Nanomaterials- Carbon Nanotubes						

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

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:	Super Growth SWCNT

Test material form

aerosol

Details on test material

SWCNT aerosol exposure chamber for the inhalation study

Results and discussions

Any other information on results incl. tables

Width = 0.21 μm (SD = 1.7)

Length = 0.69 μm (SD = 1.7)

Overall remarks, attachments

Illustration (picture/graph)

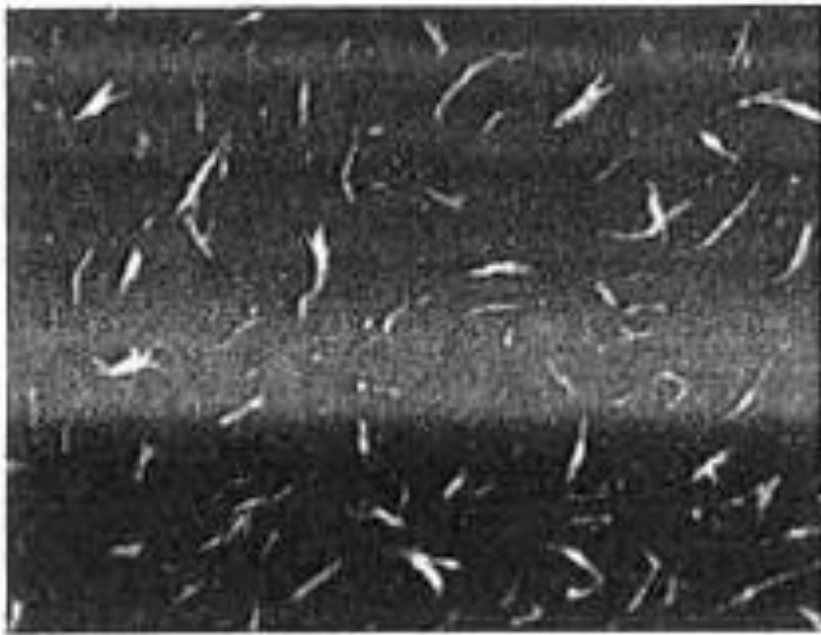


Fig. 2. AFM image of SWCNTs in testing solution,

Applicant's summary and conclusion

Executive summary

Average Diameter of tubes=8.2nm (SD=1.7),
Average Length of tubes=0.23 μm (SD=1.8),measured by TEM
Samples in testing solution;
Tube diameter; 12.0 $\pm$ 6.5nm(mean $\pm$ standard deviation),
Length;0.51 $\pm$ 1.6 μm , measured by AFM

4.6 Vapour pressure

4.7 Partition coefficient

4.8 Water solubility

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**4.25 Crystalline phase*****Endpoint study record: Nikkiso SWCNT Crystalline phase.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**

other: Raman Spectrul Analysis

Principles of method if other than guideline

The soundness of the structure (Defect of graphene sheet) can be seen by the ratio of G-band and D-band of Raman Spectrum.

Test materials**Test material identity**

Identifier	Identity
	Nikkiso SWCNT

Results and discussions**Crystallographic composition**

G/D = 116 +/- 21

Endpoint study record: Super-Growth Crystalline phase.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
	AIST	2011	Risk assessment report of the nanomaterial – carbon nanotubes –	NEDO Project (P06041)					
	Y. Morimoto, M. Hirohashi, N. Kobayashi, A. Ogami, M. Horie, T. Oyabu, T. Myojo, M. Hashiba, Y. Mizuguchi, T. Kambara, B. W. Lee, E. Kuroda, M. Shimada, W. N. Wang, K. Mizuno, K. Yamamoto, K. Fujita, J. Nakanishi, I. Tanaka	2012	Pulmonary toxicity of well-dispersed single-wall carbon nanotubes after inhalation	Nanotoxicology Nov. 2012, Vol. 6, No. 7, Pages 766-775 (doi:10.3109/17435390.2011.620719)					

Materials and methods

Method

other: Raman Spectral Analysis

Principles of method if other than guideline

The soundness of the structure (Defect of graphene sheet) can be seen by the ratio of G-band and D-band of Raman Spectrum.

Test materials

Test material identity

Identifier	Identity
	Super- Growth

Results and discussions

Crystallographic composition

G/D = 9.4 (SD=3.4)

4.26 Crystallite and grain size

4.27 Aspect ratio/shape

4.28 Specific surface area

Endpoint study record: Nikkiso SWCNT 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 ISO

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 Manufactured Nanomaterial -Carbon Nanotubes-	NEDO Project (06041)					

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 SWCNT

Results and discussions

Specific Surface Area

Specific Surface Area m²/g

Endpoint study record: Super Growth SWCNT 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 ISO

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 Manufactured Nanomaterials -Carbon Nanotubes-	NEDO Project (P06041)	AIST				

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
	Super Growth SWCNT

Results and discussions**Specific Surface Area**

Specific Surface Area 1064 m²/g

4.29 Zeta potential

Endpoint study record: Nikkiso SWCNT Zeta potential.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	AIST	2011	Risk assessment report of the nanomaterial – carbon nanotubes –	NEDO Project (P06041)					

Materials and methods

Methods

Electrophoresis

Test materials

Test material Identity

Identifier	Identity
	Nikkiso SWCNT
	Nikkiso SWCNT

Any other information on materials and methods incl. tables

Zeta potential of the solution used for in vitro study was measured.
(DMEM-FBS 10%)

Results and discussions**Zeta Potential**

Zeta Potential ca. -19.1 mV

Endpoint study record: Super GrowthZeta potential.001**Administrative Data**

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies Measured 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**

Electrophoresis

Test materials

Test material Identity

Identifier	Identity
	Super Growth
	Super Growth

Any other information on materials and methods incl. tables

Zeta potential of the solution used for in vitro test was measured.
(DMEM-FBS)

Results and discussions

Zeta Potential

Zeta Potential ca. -14.7 mV

Standard Deviation 0.9

4.30 Surface chemistry

Endpoint study record: Nikkiso SWCNT Surface chemistry.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies Measured 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
	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

The material is not surface treated. However, in order to check the absence of functional groups and the defect density by determining the organic carbon content and the oxygen content.

Test materials**Test material Identity**

Identifier	Identity
	Nikkiso SWCNT

Results and discussions**Functionalisation****Remarks on results incl. tables**

O/C = 0.022

Endpoint study record: Super Growth Surface chemistry.001**Administrative Data**

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

The material is not surface treated. However, in order to check the absence of functional groups and the defect density by determining the organic carbon content and the oxygen content.

Test materials**Test material Identity**

Identifier	Identity
	Super Growth

Results and discussions**Functionalisation****Remarks on results incl. tables**

O/C = 0.003

4.31 Dustiness

Endpoint study record: Super Growth 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 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
	AIST	2011	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

Principles of method if other than guideline

Maynard A. et al. developed a dustiness measurement method with the vortex shaker which requires a small amount of sample.

Test materials

Test material Identity

Identifier	Identity
	Super Growth

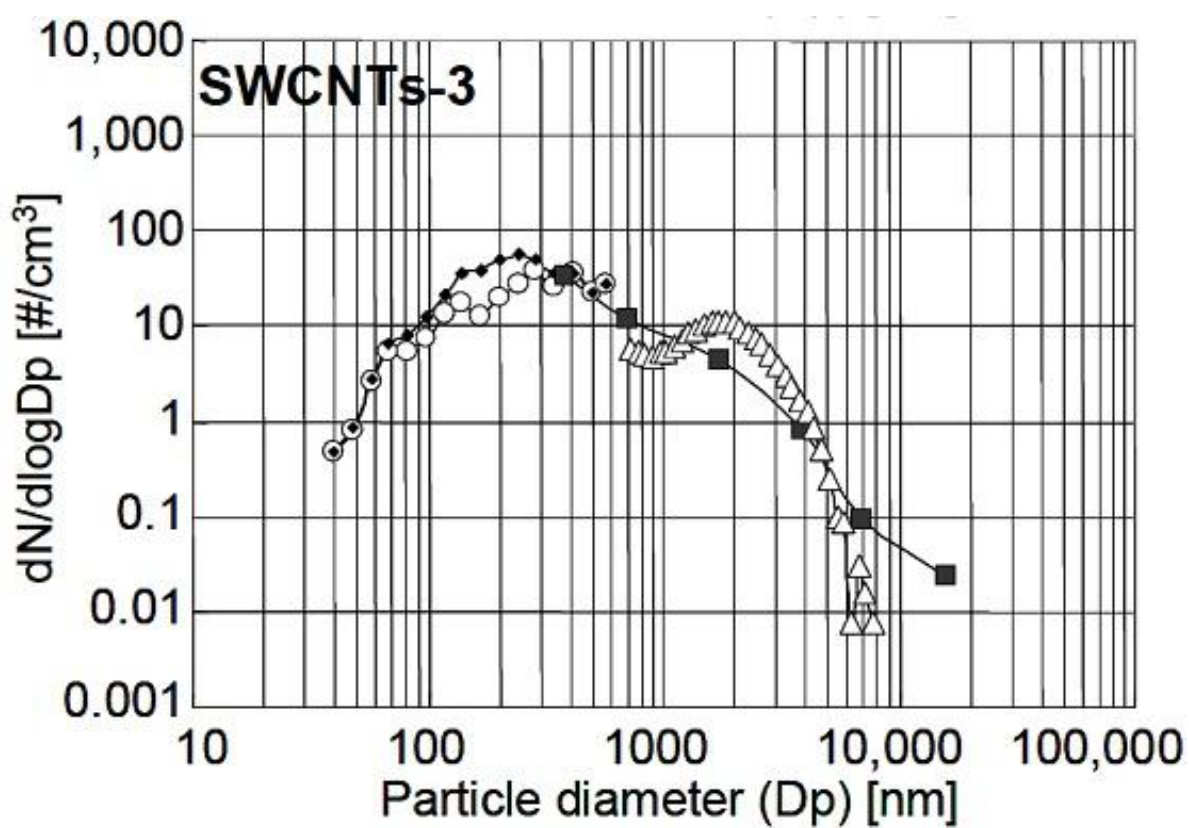
Results and discussions

Dustinex Index

Mean ca. 0.025 mg/m³

Overall remarks, attachments

Illustration (picture/graph)



4.32 Porosity

Endpoint study record: Nikkiso SWCNT 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 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					

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 SWCNT

Results and discussions

Specific pore volume

Mean ca. 30.5 cm³/g

Remarks on results incl. tables

Pore volume =: 30.5 ml/g

Davg = 190 nm

Endpoint study record: Super Growth 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 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					

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
	Super Growth

Results and discussions**Specific pore volume**

Mean ca. 18.6 cm³/g

Remarks on results incl. tables

Pore volume = 18.6 ml/g

Davg = 54nm

4.33 Pour density**Administrative Data**

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

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		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

Test materials

Test material Identity

Identifier	Identity
	Super Growth

Results and discussions

Pour Density

Mean ca. 0.0192 g/cm³

4.34 Photocatalytic activity

Endpoint study record: Nikkiso SWCNT 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

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		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: ISO22197-1 / JIS R1701	

Test materials**Test material Identity**

Identifier	Identity
	Nikkiso SWCNT

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 SWCNT		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%

Endpoint study record: Super Growt 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

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		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
	Super Growth

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					
	Super-Growth		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

5.1.1 Phototransformation in air

5.1.2 Hydrolysis

Endpoint study record: Dispersion Stability in water of US 4 SWCNTs: Hydrolysis.001

Administrative Data

other:OECD/WPMN

Purpose flag supporting study

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Zepp R.	2012							

Materials and methods

Test materials

Test material identity

Identifier	Identity
other:	Underivatized SWCNT (NIST SRM 2483)
other:	Carbon Solutions Inc
other:	NanoIntegris Inc
other:	Cheap Tubes, Inc

Details on test material

Single-Wall Carbon Nanotubes, black powder, insoluble, pH 4-10.

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

Surfactants (sodium dodecylsulfate SDS, and sodium dodecyl benzene sulfonate SDBS), polymer (polyvinylpyrrolidone PVP, 360 kDa), and a model NOM, Suwannee river humic acid SRHA (2S101H, IHSS) to disperse the SWCNTs. Dispersion followed using fluorescence in near IR as well as UV-visible spectroscopy. These were dispersed in aqueous media by sonication in the presence of natural organic matter, biomacromolecules, polymers and surfactants. UV/visible/near infrared spectra were measured as well as fluorescence excitation/emission matrix spectra. Results showed that while SWCNT dispersibility varied, the NIST sample in general had the greatest dispersibility followed by Carbon Solutions SWCNTs, and then the CheapTubes SWCNTs.

Endpoint study record: US CheapTubes Dispersion Stability in water.001**Administrative Data**

Purpose flag	supporting study		
Study result type	experimental result	Study period	2011
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Testing was completed under a U.S. EPA, Office of Research and Development research plan including a quality assurance project plan and in accordance with EPA QA/QC guidance. Data collected using techniques similar to prior published works.		

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Bouchard et al.	2012	Aggregation Kinetics and Transport of Single-Walled Carbon Nanotubes at Low Surfactant Concentrations	Environ. Sci. and Technol. 46 (8), 4458-4465					

Materials and methods

GLP compliance

no

Test materials

Details on test material

SWNTs from Cheap Tubes Inc. (Brattleboro, VT, USA); 90% wt. purity, outside diameter 1.1 nm

Study design

Details on test conditions

SWNTs from Cheap Tubes have been used in a number of environmental studies. In this study, SWNTs were added to sodium dodecyl sulphate (SDS) 0.10, 0.05, 0.01, and 0.001 % (w/v) solutions then sonicated with a probe sonicator for 10 min at an energy level of ~30 W. The SWNT suspensions were then centrifuged at 40,000 RCF for 4 h to remove impurities and minimize the presence of bundled SWNTs. The supernatants were then carefully decanted off to form the working stock suspensions which were stored at room temperature for the duration of the experiments. These studies did not involve organism exposures; duration of aggregation and stability studies varied from < 1 min to 6 weeks. There are no OECD guidelines for the measurements made in this study; however, most of the methods used are citable in prior studies by the author and other researchers. This work is relevant to 1. protocols for aggregation of nanoparticles under environmental conditions, and fate and transport of nanoparticles in the surface and subsurface. Time-resolved dynamic light scattering was used to measure the change of SWNT intensity-averaged hydrodynamic diameter (Dh) with time and as a function of background solution SDS concentrations and ionic strength (Ca and Na). The initial aggregation period was defined as the time period from experiment initiation (t0) to the time when measured Dh values exceeded 1.50Dh, initial. To determine the long term stability of the SDS-SWNTs in representative environmental systems, the suspended mass concentrations of SWNTs in 0.01 and 0.001% (w/v) SDS and in 10 mM NaCl, 1.0 mM CaCl2 and in Calls Creek water, a small stream near Athens GA, USA, were measured over a 6-week period. Column transport studies were conducted under saturated conditions using a fresh water sediment. SWNT aggregation behavior was investigated over a wide range of ionic strengths (ex: 0.001 - 1.0 M NaCl) as well as in a surface water: pH 7.9, conductivity 26 μ S / cm, total dissolved solids 27 ± 5.2 mg/L, and total organic carbon (TOC) 2.8 ± 0.57 mg/L) after filtration through a 0.45 μ m filter. The sediment used mainly contained quartz, small amounts of biotite, albite, orthoclase, and a very minor abundance of hematite, as identified by X-ray diffraction, as well as amorphous Al hydroxides (i.e., 146 mg/kg hydroxylamine extractable Al). The sediment also had 0.057% (by weight) organic carbon, 10 meq/kg cation exchange capacity, and 0.789 m²/g BET surface area (roughness factor 126).

Duration of test

6 wk

Results and discussion

Any other information on results incl. tables

The critical coagulation concentration (CCC) values increased with SDS concentration for the Na+

treatments, but the Ca²⁺ treatments were less sensitive to SDS concentration changes. Longer term stability studies with SDS concentrations orders of magnitude below the SDS critical micelle concentration demonstrated that SWNTs remained suspended for over six weeks in a surface water. Transport studies in a freshwater sediment similarly showed a SDS concentration-dependent mobility of SDS-wrapped SWNTs in that SWNTs showed a relatively greater retention at lower SDS concentrations (0.001%-0.05% w/v) than at a higher SDS concentration (0.1%). It is hypothesized that the stability and mobility of SWNT suspensions is directly related to the surface coverage of SDS on the SWNT surface that simultaneously increases electrosteric repulsion and decreases surface chemical heterogeneity.

Overall remarks, attachments

Remarks on results including tables and figures

Overall, these studies demonstrate that low levels of surfactant are effective in stabilizing and mobilizing SWNTs in environmental media. The SDS-SWNT systems studied demonstrated that at SDS concentrations more than two orders of magnitude below the CMC and within the range of surfactants in municipal sewer discharges 58, SWNT can be dispersed at concentrations > 10 mg/L. The stability of SDS wrapped SWNT in a representative surface water points to the possibility of long residence times in the water column; however, retention of SDS-SWNTs by sediment indicates that transport of these nanomaterials would be limited in soils, sediments, and other natural porous media.

5.1.3 Phototransformation in water

Endpoint study record: Phototransformation of US 4 SWCNTs in water.001

Administrative Data

other:OECD/WPMN

Purpose flag supporting study

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Zepp R.	2013			US EPA				

Materials and methods**Test materials*****Test material identity***

Identifier	Identity
other:	Underivatized SWCNT (NIST SRM 2483)
other:	Carbon Solutions Inc
other:	NanoIntegris Inc
other:	Cheap Tubes, Inc

Details on test material

Single-Wall Carbon Nanotubes, black powder, insoluble, pH 4-10.

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

Underivatized SWCNT (NIST SRM 2483). Richard Zepp. Photochemical data for SWCNT in presence of surfactants and Suwannee River humic acid obtained. Fluorescence in near IR as well as UV visible spectroscopy used to follow direct photoreaction. The four SWCNT that were tested exhibited distinct characteristics in band gap absorbance and fluorescence, indicating that the SWCNT samples are enriched in varied chiralities. Upon light exposure ($\lambda \geq 300$ nm), the band gap absorbance and fluorescence of NIST SWCNTs disappeared in the presence of H_2O_2 , but not in the presence of Rose Bengal (O_2 generator) or in suspensions of SWCNT alone. This result indicates that SWCNTs can be photochemically altered in the environment when sources of hydroxyl radicals are present.

5.2 Biodegradation

5.2.1 Biodegradation in water: screening tests

Endpoint study record: Super Growth SWCNT Biodegradation in water: Modified MITI Test (I)

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted Under GLP 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	Biodegradation test of Super Growth SWCNT		CERI(Cheical Evaluation and Research Institute, Japan)	15652			

Materials and methods

Test type

ready biodegradability

Test guideline

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

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:	Super Growth SWCNT

Study design**Oxygen conditions**

anaerobic

Inoculum or test system

sewage, domestic, non-adapted

Duration of test (contact time)

>= 28 d

Initial test substance concentration

Initial conc.	Based on
>= 100 mg/L	

Details on study design

Bottle 1: test substance in water

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

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

Bottle 6: inoculum in mineral medium

Temperature: 25+/-1C , under dark, stirring during test period (28 days)

Activated sludge 30 mg/L

Test Item: Biochemical oxygen demand (BOD) and Dissolved organic carbon (DOC)

Results and discussions**Details on results**

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 62 % and 66 % (Aniline, after 7 days and 14 days, respectively) DOC due to test substance: < 0.6 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)	1.6	-7.9	-7.8	-7.8	79.8
DOC (mg-C)	0	-0.5	0	-0.5	30
DOC detect ratio (%)	0	-2	0	-2	--

Degradation (BOD) in 28 days

	Sludge + Test sample			
Sample No.	(2)	(3)	(4)	average
Degradation (%)	-10	-10	-10	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)	Water + test material	0.0	0.6	1.6	1.6
(2)	Sludge + test material	0.0	1.0	2.5	2.5
(3)	Sludge + test material	0.0	1.1	2.6	2.6
(4)	Sludge + test material	0.0	1.0	2.6	2.6
(5)	Control (blank)	2.8	5.6	9.8	10.4
(6)	Control (Sludge + aniline)	58.9	65.5	69.9	70.6

Overall remarks, attachments

Illustration (picture/graph)

Study No. 15652 (Test item 物質C)

Cultivation conditions:

Concentration

Test item 100 (mg/L)

Reference item (aniline) 100 (mg/L)

Activated sludge 30 (mg/L)

Temperature 25 ± 1 °C

Duration 28days (Feb.09,2011 - Mar.09,2011)

Note: —

Vessel No.	Sample Description	BOD (mg)			
		7th day	14th day	21st day	28th day
[1]	Water + test item	0.0	0.6	1.6	1.6
[2]	Sludge + test item	0.0	1.0	2.5	2.5
[3]	Sludge + test item	0.0	1.1	2.6	2.6
[4]	Sludge + test item	0.0	1.0	2.6	2.6
[5]	Control blank [B]	2.8	5.6	9.8	10.4
[6]	Sludge + aniline	58.9	65.5	69.9	70.6

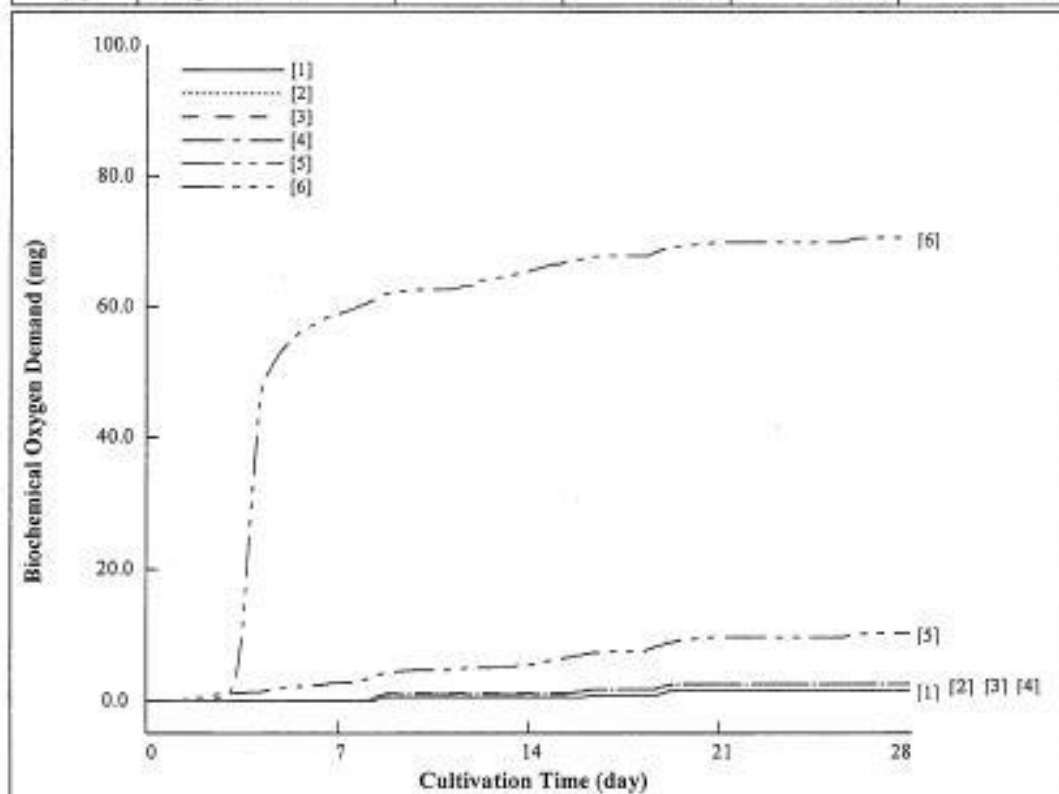


Fig. 1 Chart of BOD.

Mar.09,2011

Name

A. Takemura

Applicant's summary and conclusion**Conclusions**

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

Endpoint study record: Nikkiso SWCNT Biodegradation in water: Modified MITI Test (I)**Administrative Data**

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted Under GLP 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	Biodegradation test of Nikkiso SWCNT		CERI(Chemical Evaluation and Research Institute, Japan)	15618			

Materials and methods**Test type**

ready biodegradability

Test guideline

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

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 SWCNT

Study design

Oxygen conditions

anaerobic

Inoculum or test system

sewage, domestic, non-adapted

Duration of test (contact time)

>= 28 d

Initial test substance concentration

Initial conc.	Based on
>= 100 mg/L	

Details on study design

Bottle 1: test substance in water

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

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

Bottle 6: inoculum in mineral medium

Temperature: 25+/-1C , under dark, stirring during test period (28 days)

Activated sludge 30 mg/L

Test Item: Biochemical oxygen demand (BOD) and Dissolved organic carbon (DOC)

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 54 % and 70 % (Aniline, after 7 days and 14 days, respectively) DOC due to test substance: < 0.6 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)	1.1	0.4	0.2	0.1	79.8
DOC (mg-C)	0	0	0.6	0	30
DOC detect ratio (%)	0	0	2	0	--

Degradation (BOD) in 28 days

	Sludge + Test sample			
Sample No.	(2)	(3)	(4)	average
Degradation (%)	0	0	0	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	0.5	1.1
(2)	Sludge + test material	0.7	3.3	4.7	6.0
(3)	Sludge + test material	0.5	3.0	4.4	5.8
(4)	Sludge + test material	0.4	3.1	4.4	5.7
(5)	Control (blank)	1.3	2.9	4.1	5.6
(6)	Control (Sludge + aniline)	50.4	66.2	69.9	72.2

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

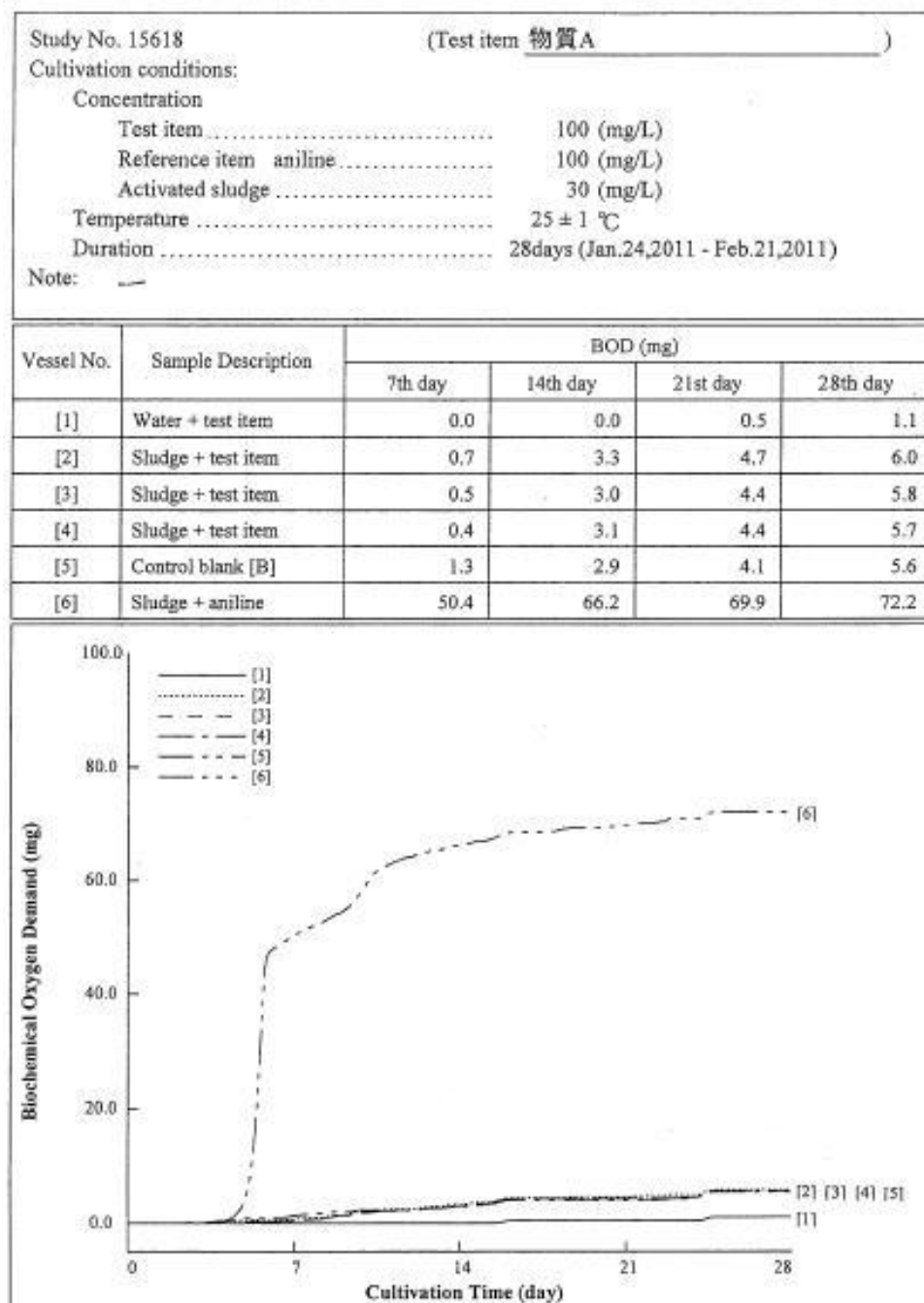


Fig. 1 Chart of BOD.

Feb.21,2011 Name

A. Taberna

Applicant's summary and conclusion

Conclusions

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

Endpoint study record: Nikkiso SWCNT Biodegradation in water: Manometric Respirometry Test

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted Under GLP 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	Manometric Respirometry test of Nikkiso SWCNT		CERI(Chemical Evaluation and Research Institute, Japan)	15606			

Materials and methods

Test type

ready biodegradability

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test)	no

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 SWCNT

Study design

Oxygen conditions

anaerobic

Inoculum or test system

other: Returned sludge of a sewage treatment plant (Kurume city, Japan)

Duration of test (contact time)

≥ 28 d

Initial test substance concentration

Initial conc.	Based on
≥ 100 mg/L	

Details on study design

Bottles 1 & 2: containing and incolums(activated sludge): test suspension

Bottles 3 & 4: containing only inoculum: inoculum blank

Bottle 5: reference compound (aniline) and inoculum:procedure control

Temperature: $22 \pm 1^\circ\text{C}$, under dark, stirring during test period (28 days)

Activated sludge 30 mg/L,

Test substance and reference substance 100mg/L

Test Item: Biochemical oxygen demand (BOD) and Dissolved organic carbon (DOC)

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 71 % (Aniline, 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***

	Sludge + Test material		Sludge only		Theoretic value
Sample No.	(1)	(2)	(3)	(4)	--
BOD (mg)	-3.6	-3.1	8.2	8.2	79.8
DOC (mg-C/L)	n.d.	n.d.	0.3	0.4	30
DOC detect ratio (%)	0	0	--	--	--

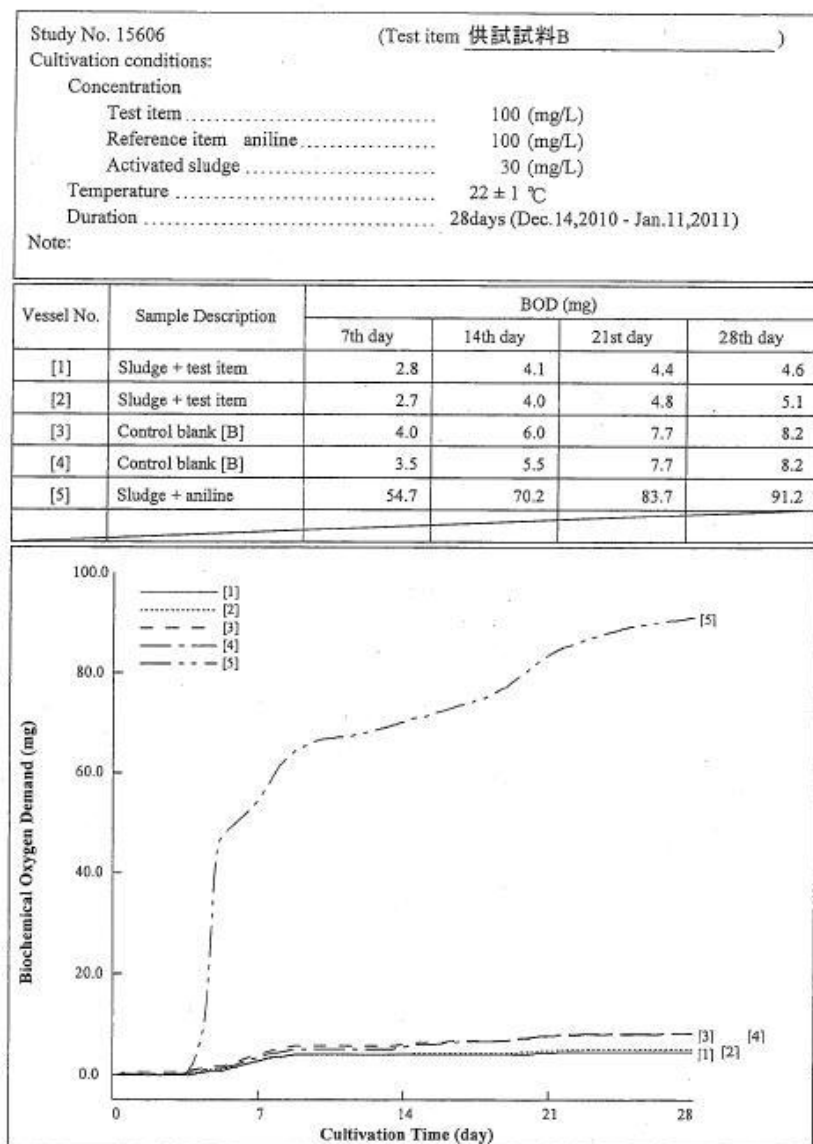
Degradation (BOD) in 28 days

	Sludge + Test sample		
Sample No.	(2)	(3)	average
Degradation (%)	-5	-4	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	2.8	4.1	4.4	4.6
(2)	Sludge + test material	2.7	4.0	4.8	5.1
(3)	Control (blank)	4.0	6.0	7.7	8.2
(4)	Control (blank)	3.5	5.5	7.7	8.2
(5)	Control (Sludge + aniline)	54.7	70.2	83.7	91.2

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

Jan.11,2011

Name

*A. T. Kuroki***Applicant's summary and conclusion****Conclusions**

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

Endpoint study record: Super Growth SWCNT Biodegradation in water: Manometric Respirometry Test

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted Under GLP 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	Manometric respirometry test of Super Growth SWCNT		CERI(Chemical Evaluation and Research Institute, Japan)	15607			

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:	Super Growth SWCNT

Study design

Oxygen conditions

anaerobic

Inoculum or test system

other: Returned sludge of a sewage treatment plant (Kurume city, Japan)

Duration of test (contact time)

≥ 28 d

Initial test substance concentration

Initial conc.	Based on
≥ 100 mg/L	

Details on study design

Bottles 1 & 2: containing and incolums(activated sludge): test suspension

Bottles 3 & 4: containing only inoculum: inoculum blank

Bottle 5: reference compound (aniline) and inoculum:procedure control

Temperature: $22 \pm 1^\circ\text{C}$, under dark, stirring during test period (28 days)

Activated sludge 30 mg/L,

Test substance and reference substance 100mg/L

Test Item: Biochemical oxygen demand (BOD) and Dissolved organic carbon (DOC)

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 73% (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

	Sludge + Test material		Sludge only		Theoretic value
Sample No.	(1)	(2)	(3)	(4)	--
BOD (mg)	-0.9	-1.8	5.3	5.3	79.8
DOC (mg-C/L)	n.d.	0.3	0.4	0.4	30
DOC detect ratio (%)	0	0	--	--	--

Degradation (BOD) in 28 days

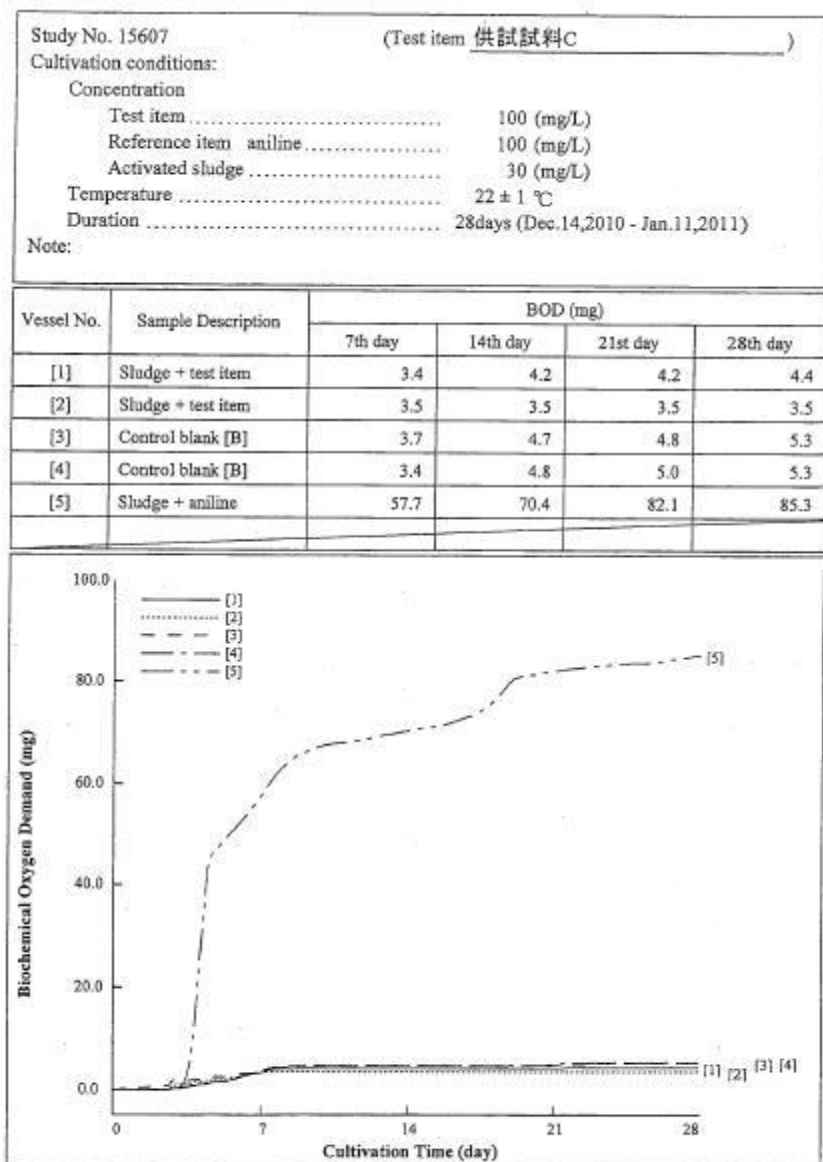
	Sludge + Test sample		
Sample No.	(1)	(2)	average
Degradation (%)	-1	-2	0(*)

(*) 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	3.4	4.2	4.2	4.4
(2)	Sludge + test material	3.5	3.5	3.5	3.5
(3)	Control (blank)	3.7	4.7	4.8	5.3
(4)	Control (blank)	3.4	4.8	5.0	5.3
(5)	Control (Sludge + aniline)	57.7	70.4	82.1	85.3

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



Jan.11,2011

Name

A. T. K. K.

Applicant's summary and conclusion

Conclusions

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

Endpoint study record: Super Growth SWCNT Biodegradation in water: Inherent Biodegradability Test

Administrative Data

other:OECD/WPMN

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

The test was conducted Under GLP 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	Biodegradation test of Super Growth SWCNT		CERI(Chemical Evaluation and Research Institute, Japan)	15653			

Materials and methods

Test type

ready 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
other:	Super Growth SWCNT

Study design

Oxygen conditions

anaerobic

Inoculum or test system

activated sludge, non-adapted

Details on inoculum

Activated sludge prepared according to the TG 301C

Duration of test (contact time)

≥ 28 d

Initial test substance concentration

Initial conc.	Based on
≥ 100 mg/L	

Details on study design

Bottle 1: test substance in water

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

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

Bottle 6: inoculum in mineral medium

Temperature: $25 \pm 1^\circ\text{C}$, under dark, stirring during test period (28 days)

Activated sludge 30 mg/L

Test Item: Biochemical oxygen demand (BOD) and Dissolved organic carbon (DOC)

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 57% and 68% (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)	0.3	-9.2	-4.7	-6.0	23.9
DOC (mg-C/L)	0	-0.5	0.2	-0.5	9
DOC detect ratio (%)	0	-5	2	-5	--

Degradation (BOD) in 28 days

	Sludge + Test sample			
Sample No.	(2)	(3)	(4)	average
Degradation (%)	-38	-20	-25	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	0.1	0.3
(2)	Sludge + test material	3.9	6.2	7.4	8.3
(3)	Sludge + test material	6.0	9.0	11.3	12.8
(4)	Sludge + test material	7.2	8.9	10.7	11.5
(5)	Control (blank)	7.1	12.7	15.7	17.5
(6)	Control (Sludge + aniline)	54.0	65.0	69.4	70.6

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

Study No. 15653	(Test item 物質C)
Cultivating conditions:	
Concentration	
Test item	30 (mg/L)
Reference item (aniline)	100 (mg/L)
Activated sludge	100 (mg/L)
Temperature	25 ± 1 °C
Duration	28 days (Feb.9,2011 - Mar.9,2011)
Note: Activated sludge 30 mg/L (Sludge + aniline)	

Vessel No.	Sample Description	BOD (mg)			
		7th day	14th day	21st day	28th day
[1]	Water + test item	0.0	0.0	0.1	0.3
[2]	Sludge + test item	3.9	6.2	7.4	8.3
[3]	Sludge + test item	6.0	9.0	11.3	12.8
[4]	Sludge + test item	7.2	8.9	10.7	11.5
[5]	Control blank [B]	7.1	12.7	15.7	17.5
[6]	Sludge + aniline	54.0	65.0	69.4	70.6

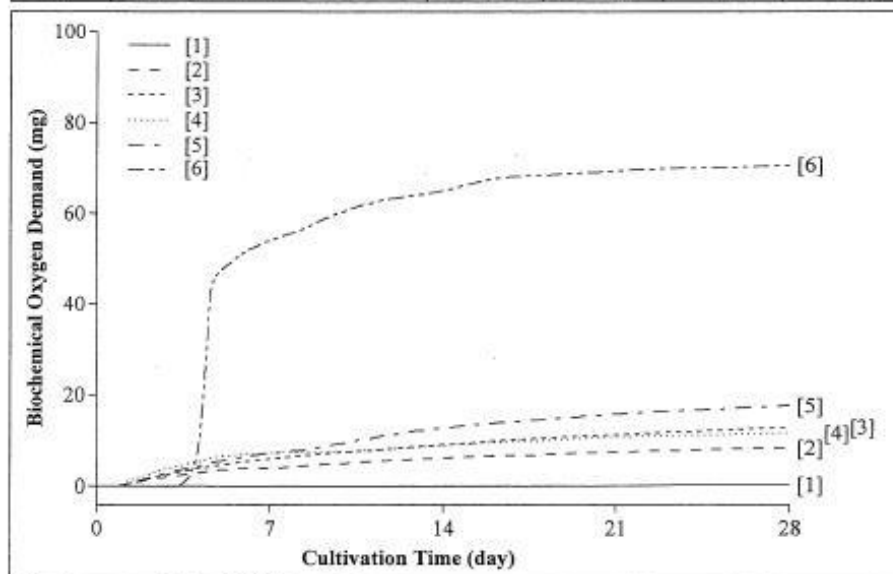


Fig. 1 Chart of BOD.

Mar.9,2011

Name

*A. Takemura***Applicant's summary and conclusion****Conclusions**

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

Endpoint study record: Nikkiso SWCNT Biodegradation in water: Inherent Biodegradability Test

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted Under GLP 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	Biodegradation test of Nikkiso SWCNT		CERI(Chemical Evaluation and Research Institute, Japan)	15619			

Materials and methods

Test type

ready 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
other:	Nikkiso SWCNT

Study design

Oxygen conditions

anaerobic

Inoculum or test system

activated sludge, non-adapted

Details on inoculum

Activated sludge prepared according to the TG 301C

Duration of test (contact time)

≥ 28 d

Initial test substance concentration

Initial conc.	Based on
≥ 100 mg/L	

Details on study design

Bottle 1: test substance in water

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

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

Bottle 6: inoculum in mineral medium

Temperature: $25 \pm 1^\circ\text{C}$, under dark, stirring during test period (28 days)

Activated sludge 30 mg/L

Test Item: Biochemical oxygen demand (BOD) and Dissolved organic carbon (DOC)

Results and discussions

Details on results

Averaged percent biodegradation based on BOD: 0 % (Test substance, after 28 days), 65% 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)	1.5	-2.3	-4.6	-3.9	23.9
DOC (mg-C/L)	0	-0.6	-0.6	-0.6	9
DOC detect ratio (%)	0	-7	-7	-7	--

Degradation (BOD) in 28 days

	Sludge + Test sample			
Sample No.	(2)	(3)	(4)	average
Degradation (%)	-10	-19	-16	0(*)

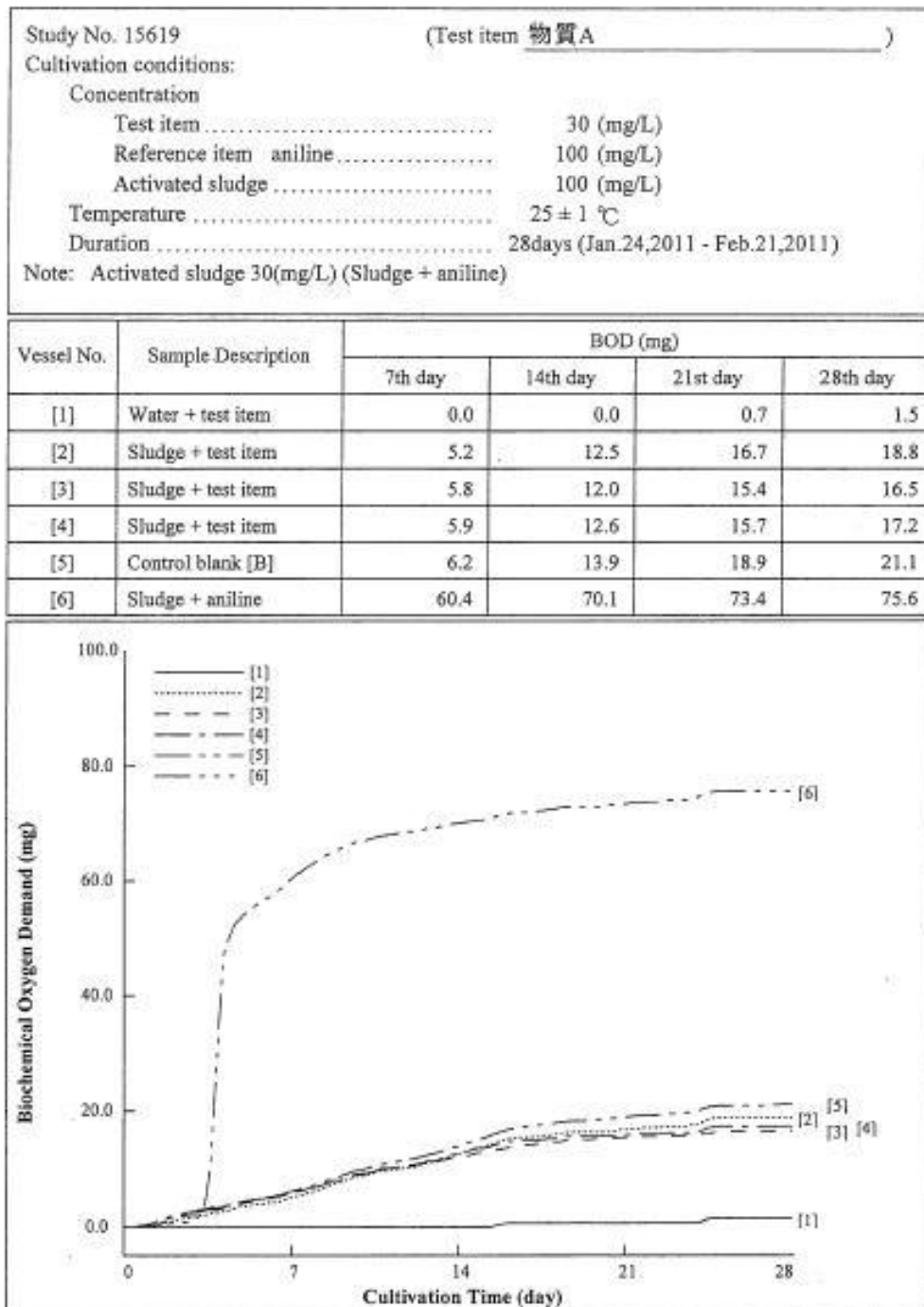
() The average value became negative, so the value set as 0*

Change in BOD

Sample No.	Sample description	BOD (mg)			
		7 th day	14 th day	21 st day	28 th day
(1)	Water + test material	0.0	0.0	0.7	1.5
(2)	Sludge + test material	5.2	12.5	16.7	18.8
(3)	Sludge + test material	5.8	12.0	15.4	16.5
(4)	Sludge + test material	5.9	12.6	15.7	17.2
(5)	Control (blank)	6.2	13.9	18.9	21.1
(6)	Control (Sludge + aniline)	60.4	70.1	73.4	75.6

Overall remarks, attachments

Illustration (picture/graph)



Feb.21,2011

Name

A. Taperal

Applicant's summary and conclusion

Conclusions

The result indicated that the test substance is not readily 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

5.3.1 Bioaccumulation: aquatic / sediment

*Endpoint study record: Transfer Marine System1;SWeNT
SWCNT;Bioaccumulation: aquatic / sediment.002*

Administrative Data

other:OECD/WPMN

Purpose flag supporting study

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Diamond S., Ho K.	2013			US EPA				

Materials and methods

Test materials

Test material identity

Identifier	Identity
other:	Southwest Nanotechnologies (SWeNT) SG65 SWCNT

Details on test material

Weight: 85-99% SWCNT, Diameter Given from Manufacturer: 0.8 +/- 0.1nm

Impurities: Molybdenum and Cobalt ◇

Weight: 1-15%

Single wall carbon nanotubes, with various amounts of molybdenum and cobalt, black powder, solid, insoluble to water, pH 4-10

Test conditions

Any other information on materials and methods incl. tables

Cyclotella sp (algae) and *Artemia salina* (brine shrimp) were spiked at 1 ug/g, respectively, as prey for *A. abdita* and *A. bahia*, respectively. Acute toxicity was recorded. Trophic transfer was measured using novel near infrared fluorescence (NIRF) spectroscopic method.

Overall remarks, attachments

Remarks on results including tables and figures

SWNT were not toxic to *A. abdita* or *A. bahia* at the concentrations tested after exposure through sediment or dietary routes. No bioaccumulation of SWNT was observed. No toxicity was observed for *A. abdita* or *A. bahia* at any concentration.

Endpoint study record: Transfer Marine System2; SWeNT SWCNT; Bioaccumulation: aquatic / sediment.002

Administrative Data

other:OECD/WPMN

Purpose flag supporting study

Study result type experimental result

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Diamond S., Ho K.	2013			US EPA				

Materials and methods**Test materials****Test material identity**

Identifier	Identity
other:	Southwest Nanotechnologies (SWeNT) SG65 SWCNT

Details on test material

Weight: 85-99% SWCNT,

Diameter Given from Manufacturer: 0.8 +/- 0.1nm

Impurities: Molybdenum and Cobalt ◇

Weight: 1-15%

Single wall carbon nanotubes, with various amounts of molybdenum and cobalt, black powder, solid, insoluble to water, pH 4-10

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

Examined trophic transfer and uptake through a simplified marine food chain. Southwest Nanotechnologies SWNT SG65 were added to marine sediments as well as spiked into *Isochrysis sp* (algae). The spiked algae were fed to *Mercenaria mercenaris* (clam) which were ultimately fed to *Nereis virens* (worm).

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

Data for the simplified marine food chain is currently under analysis. Preliminary results indicate no uptake or trophic transfer of SWNT through this food chain.

Endpoint study record: US RTI SWCNT Bioaccumulation: aquatic / sediment.001**Administrative Data**

Purpose flag	supporting study		
Study result type	experimental result	Study period	2009-2011
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	Research performed based on established U.S. EPA procedures and published methods		

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Parks et al.		Bioaccumulation and toxicity of single walled carbon nanotubes (SWNT) to benthic organisms at the base of the marine food chain	Environmental Toxicology and Chemistry					

Materials and methods**Principles of method if other than guideline**

U.S. EPA. 1993. Bedded Sediments Bioaccumulation Tests. EPA-600-R-93-183. Office of Research and Development, Washington DC.

U.S. EPA. 2007. Sediment Toxicity Identification Evaluation (TIE) Phases I, II and III: Guidance Document. EPA-600-R-07-080. Office of Research and Development, Washington DC.

Ho KT, Kuhn A, Pelletier MC, McGee F, Burgess RM, Serbst JR. 2000. Sediment toxicity assessment: comparison of standard and new testing designs. Arch Environ Contam Toxicol 39:462-468.

Measurement of non-labelled SWCNT followed methods described in Schierz et al. (2013): Schierz A, Parks AN, Washburn KM, Chandler GT, Ferguson PL. 2013. Characterization and quantitative analysis of single-walled carbon nanotubes in the aquatic environment using near-infrared fluorescence spectroscopy. Environ Sci Technol 46:12262–12271.

GLP compliance

yes

Test materials**Details on test material**

1. Non-labelled SWCNT Toxicity Testing and Bioaccumulation Studies: SWCNT were CoMoCAT nanotubes (SG65, SG76, CG100) manufactured by SouthWest NanoTechnologies, Inc (SWeNT) (Norman, Oklahoma, USA) and a preliminary proposed standard reference material SWCNT provided by the Organization for Economic Cooperation and Development (OECD). SWeNT products were characterized, high purity mixtures of SWCNT with either narrow diameter distributions and specificity (e.g., SG65, SG76) or relatively broad diameter distributions (CG100). Stable suspensions of SWCNT were characterized by preparation in 2% w/v sodium deoxycholate (SDC) using 10 minutes of sonication (high energy) at 50% amplitude in a salt-water ice bath followed by centrifugation at 17,860 x g and 22°C for 30 minutes. This procedure removed non-suspended SWCNT bundles. For toxicity testing and bioaccumulation studies, suspensions of the unlabeled SWCNT materials were prepared at 1 mg SWCNT/mL 2% w/v SDC without the centrifugation step. If stock suspensions were not used immediately after preparation, before re-use, stock suspensions were sonicated prior to addition of any amendment to testing media (e.g., sediment or food). Exposure concentrations were 0.1, 1 and 10 ug SWCNT/g test media. Quantification of SWCNT in water samples, sediments, fecal pellets, and tissues was performed using near-infrared fluorescence (NIRF) spectroscopy following extraction with a 2% sodium deoxycholate solution.
2. 14C-Labelled SWCNT Bioaccumulation Studies: 14C-labelled SWCNT was generated using the arc-discharge method (Research Triangle Institute, Durham, North Carolina, USA). Purification was conducted using nitric acid to introduce oxygen containing groups such as carboxyl to defective and end sites. The 14C-labelled SWCNT stock was 1.15 mg 14C-labelled SWCNT/mL deionized water (activity of 1.63 µCi/mL). Before use, the stock was resonicated for 10 minutes at 80-100 watts. Exposure concentrations were 10 and 100 ug 14C-labelled SWCNT/g test media. Quantification of 14C-labelled SWCNT associated with water samples, sediments, fecal pellets, and tissues was performed using standard scintillation counting following extraction with scintillation fluid and other extraction agents.

Test organisms***Details on test organisms***

1. Non-labelled SWCNT Toxicity Testing and Bioaccumulation Studies: 48 hour-old cultured *Americamysis bahia* (mysid) and 0.7 to 1.0 mm field collected *Ampelisca abdita* (amphipod)
2. 14C-Labelled SWCNT Bioaccumulation Studies: Adult and juvenile cultured *Leptocheirus plumulosus* (amphipod)

Test conditions

Details on test conditions

1. Non-labelled SWCNT Toxicity Testing and Bioaccumulation Studies: Ten individuals of each organism species (*A. abdita*, *A. bahia*) were added to test chambers containing 20 g wet sediment and 60 mL of 30‰ reconstituted seawater (RSW). RSW was prepared by combining 100‰ brine with deionized water. Chambers were aerated and static in a 20 °C incubator with a light cycle of 16h:8h light:dark.
2. 14C-Labelled SWCNT Bioaccumulation Studies: Chambers each contained 20 adult amphipods and 50 juvenile amphipods. Exposures included 35 mL of sediment slurry (0.1106 g dry/mL slurry) and 150 mL of 15‰ aerated RSW held in a 25 °C incubator with a light cycle of 16h:8h light:dark.

Results and discussions

Details on results

1. Non-labelled SWCNT Toxicity Testing and Bioaccumulation Studies: Percent survival ranged from 85-100% using the amphipod (*A. abdita*) and mysid (*A. bahia*) with four pristine SWCNTs assessed at concentrations up to 10 µg SWNT/g dry sediment. Further, survival ranged from 95-100% for both species when exposed to SWCNTs via sediment and food sources. Statistical analysis using one-way analysis of variance (ANOVA) ($\alpha = 0.05$) found no significant differences in survival between the treatments and controls for either organism. In the bioaccumulation component of the study, SWCNT were not detected in extracts of the depurated or non-depurated mysids or depurated amphipods. SWCNT were measured in extracts of sediment, spiked food sources (i.e., algae *C. meneghiniana* and brine shrimp *A. salina*), and non-depurated amphipods exposed to SWCNT amended algae. However, while SWCNT were detectable in *A. abdita* tissues before depuration, after depuration, no SWCNT were measured in the amphipod.
2. 14C-Labelled SWCNT Bioaccumulation Studies: Survival of adult *L. plumulosus* ranged from 87-98% of the control treatment response. Reproduction was also apparent in all treatments. No statistically significant differences in survival from the controls were found based on ANOVA ($\alpha = 0.05$). In the 14C-labelled SWCNT bioaccumulation study, *L. plumulosus* exposed to amended sediments and algae, but not the depurated treatments, showed significantly elevated bioaccumulation relative to the control treatments. Specifically, when exposed to 14C-labelled SWCNT amended algae, a statistically significant factor of five increase in bioaccumulation resulted as compared to the control treatments. By comparison, exposure to sediment amended with 14C-labelled SWCNT resulted in a significant factor of 50 increase in bioaccumulation relative to the control treatments. Amphipod exposure to algae amended with 14C-labelled SWCNT after exposure to sediment amended with 14C-labelled SWCNT did not increase tissue concentrations suggesting that sediment exposures were more critical to causing increases in bioaccumulation than algae ingestion. Following 24 hours of depuration, tissue concentrations of 14C-labelled SWCNT decreased and organisms from treatments including the highest sediment amendments (i.e., 100 µg/g sediment + control algae, and 100 µg/g sediment + 100 µg/g algae) continued to have significantly high bioaccumulation (factor of ~5). However, although the 14C-labelled SWCNT concentrations were detectable in the amphipods, in general, bioaccumulation was low. Finally, bioaccumulation factors (BAFs) were determined for the before and after

depuration treatments. All BAFs were below 1.0, and these values decreased by about one order of magnitude after depuration. These data indicate ¹⁴C-labelled SWCNT were not bioaccumulated and that the amphipod did take-up the SWCNT through ingestion, primarily of sediments, but then eliminated them during depuration.

Overall remarks, attachments

Remarks on results including tables and figures

At the concentrations prepared for the sediment and food exposures in this study, Parks et al. showed that four pristine SWCNT and one oxidized SWCNT were not toxic to three important estuarine benthic invertebrates from the base of the marine food chain. Further, following depuration, any observed bioaccumulation of pristine or oxidized SWCNTs decreased substantially. Results reported by Parks et al. are consistent with other investigations of the bioavailability, bioaccumulation and toxicity of carbon nanotubes to aquatic invertebrates. These data indicate that SWCNT present in the environment, may be taken-up by benthic organisms but then are readily eliminated suggesting there is very limited exposure. Interestingly, different organisms used different modes of uptake of the SWCNTs. For example, it was shown that *A. abdita* accumulated SWCNT primarily via ingesting algae whereas *L. plumulosus* accumulated SWCNT via exposure to sediment.

This study showed there was little evidence of the bioavailability, bioaccumulation or toxicity of SWCNT to organisms at the base of the marine benthic food chain. However, additional research is needed. An area that requires better elucidation is assessing the potential adverse effects of SWCNTs exposed to upper food chain predators feeding on organisms at the base of the food chain resulting in an elevated dose of SWCNTs. Digestive systems of some marine species have been shown to contain fluids which may be similar to the surfactants used in this study (e.g., SDC) to extract SWCNT from environmental media. Further, the presence of SWCNT in the benthic environment could represent a risk to upper food chain organisms regardless of the lack of SWCNT adverse effects at the base of the food chain. For example, this could have implications to human health if SWCNT are able to enter higher trophic levels (e.g., economically-important fish species) relevant to human diets. In this study, measured concentrations of several SWCNTs in water, food, tissues, and sediments were conducted using a novel near infrared fluorescence (NIRF) spectroscopic method. The NIRF methodology is based on each SWCNT having a unique diameter, chiral wrapping angle, and excitation-emission spectrum. The resulting unique spectral signature allows for identification of individual types of SWCNTs. This methodology, in its automated form and using deoxycholic acid sodium salt (SDC) for the extraction and resuspension of SWCNTs from sediments, water, and tissues, is available in Schierz et al. (2013). The development of methods to detect CNTs in complex media and tissues is a significant advance for assessment of SWCNT fate and effects

5.3.2 Bioaccumulation: terrestrial

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: Nikkiso SWCNT Short-term toxicity to fish.001

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. This study was conducted under GLP using OECD Test deficiencies Guideline

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	96 hr Acute Toxicity test of Nikkiso SWCNT using Rice fish		CERI	95403			

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 SWCNT

Test organisms**Test organisms (species)**

Oryzias latipes

Study design**Test type**

static

Water media type

freshwater

Total exposure duration

96 h

Test conditions***Hardness***

Total hardness(as CaCO₃); 27mg/L

Test temperature

24+/-1 C

pH

7.3

Dissolved oxygen

>80% of Saturated Dissolved Oxygen

Nominal and measured concentrations

Test substance 10 mg/L,
Dispersing agent (HCO-40) 100 mg/L

Any other information on materials and methods incl. tables

Number of species per sex per dose: Seven fishes in each test, control or vehicle control solution
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				

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	N
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

N: Nonnal(No abnormalresponse)

Endpoint study record: Super Growth SWCNT Short-term toxicity to fish.001**Administrative Data**

other:OECD/WPMN

Purpose flag key study**Study result type** experimental result**Reliability** 1 (reliable without restriction)**Rationale for reliability incl.** This study was conducted under GLP using OECD Test Guideline deficiencies**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	96 hr Acute Toxicity test of Super Growth SWCNT using Rice fish		CERI	95407			

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:	Super Growth SWCNT

Test organisms

Test organisms (species)

Oryzias latipes

Study design

Test type

static

Water media type

freshwater

Total exposure duration

96 h

Test conditions

Hardness

Total hardness(as CaCO₃); 27mg/L

Test temperature

24+/-1 C

pH

7.3

Dissolved oxygen

>80% of Saturated Dissolved Oxygen

Nominal and measured concentrations

Test substance 10 mg/L,
Dispersing agent (HCO-40) 100 mg/L

Any other information on materials and methods incl. tables

Number of species per sex per dose: Seven fishes in each test, control or vehicle control solution
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				

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	N
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

N:(No abnormal response)

Endpoint study record: Sigma-Aldrich SWCNT Short-term toxicity to fish.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Meets generally accepted scientific standards, well documented and acceptable for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Cheng et al.	2007	Effect of carbon nanotubes on developing zebrafish (Danio rerio) embryos	Env. Tox & Chem., 26: 708-716					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	other guideline: OECD TG236(2013) Fish Embryo Acute Toxicity Test	

Test materials

Test material identity

Identifier	Identity
other: Sigma-Aldrich	

Details on test material

SWCNTs (produced by arc discharge in the presence of Co and Ni as catalysts, then purified by acid leaching and thermal oxidation) were obtained from Sigma-Aldrich. 11 nm average diameter, 0.5 – 100 µm average length, purity of 90 atom percent.

Test organisms

Test organisms (species)

other: Zebrafish embryos

Test conditions

Details on test conditions

SWCNTs were dispersed in filtered tap water by stirring with a magnetic stir bar for 30 minutes, generating six concentrations of SWCNTs (20, 40, 120, 240, and 360 mg/L). Carbon black and plain water controls were also tested, and embryos were exposed for 4 to 96 hours post fertilization.

Results and discussions

Details on results

SEM images revealed that most of the SWCNT agglomerates tested were micron-sized, while the zebrafish chorion pore size was much smaller (nanometer sized); the chorion may have served as an effective barrier to CNT penetration. Delayed hatching was found at concentrations of 120 mg/L and higher, but 99% of the exposed embryos hatched by 75 h post fertilization. Delayed hatching was attributed to cobalt and nickel catalyst contaminants used in manufacture: nominal no-effect

concentrations for hatching time were 40 ug/L for Ni and 3,840 ug/L for Co in referenced studies; concentrations were estimated (based on manufacturer's impurity descriptors) to be greater than 3 mg/L Ni and 9 mg/L Co in the study by Cheng, et al. (2007).

Applicant's summary and conclusion

Conclusions

Delayed hatching was attributed to cobalt and nickel catalyst contaminants used in manufacture.

Endpoint study record: Cheap Tubes SWCNT Short-term toxicity to fish.001

Administrative Data

Purpose flag supporting 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
publication	Smith et al.	2007	Toxicity of single walled carbon nanotubes to rainbow trout, (<i>Oncorhynchus mykiss</i>): Respiratory toxicity, organ pathologies, & other physiological effects.	Aquat.Toxicol., 82: 94-109					

Materials and methods

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: Cheap Tubes	

Details on test material

SWCNTs were obtained from Cheap Tubes, 1.1 nm mean outside diameter, 5-30 µm length, minimum 96.3% carbon; maximum impurities were Al 0.08, CL 0.41, Co 2.91, and S 0.29%.

Details on test solutions

SWCNT from Cheap Tubes was supplied as a dry powder. This was then made into a stock dispersion. However, analysis of the stock dispersion for metals revealed these impurities were below detection (< 0.1 µg/l by ICP-OES). A stock solution (1 litre) were prepared by dispersing 0.5 g/l SWCNT in 3 g/l SDS and then sonicating for 2 h in a bath-type sonicator (35 kHz frequency, Fisherbrand FB 11010, Germany). This container was also manually inverted every 20 min to redistribute the foam forming at the surface of the solution. The dispersion was confirmed using an optical absorbance method, but as a precaution the stock was sonicated again each day for 30 minutes immediately prior to dosing the tanks. This was to ensure that the pipetting into the tanks used a representative sub-sample of the stock dispersion.

Test organisms

Test organisms (species)

other: juvenile rainbow trout

Details on test organisms

Normal juvenile rainbow trout, *Oncorhynchus mykiss*, weighing 30.0 g ± 5.0 g (mean ± S.E.M., n = 180 fish).

Study design

Test type

static

Water media type

freshwater

Total exposure duration

10 d **Remarks** The protocol included sampling on day 4 to provide some information in relation to the popular use of 96 h test durations in regulatory tests, but also a longer time point (10 days) because the main interest was sub-lethal effects.

Test conditions

Test temperature

15.5 ± 0.3 °C.

pH

7.16 ± 0.01

Dissolved oxygen

83 ± 0.13 %

Salinity

The electrolyte composition of the dechlorinated Plymouth tap water used was 0.3, 0.1, and 0.4 mmol/l for Na⁺, K⁺ and Ca²⁺ respectively.

Details on test conditions

Juvenile rainbow trout was exposed to SWCNTs at concentrations of 0, 0.1, 0.25, or 0.5 mg/L. SWCNTs were brought into the aqueous phase through the use of the detergent-like solvent sodium dodecyl sulfate (SDS) and sonication. Fish were exposed for up to 10 days under semi-static conditions. Stock solution concentrations of SWCNTs were confirmed, and re-dosing with SWCNT-bearing water occurred every 12 hours (in-tank concentrations of SWCNTs could not be determined). A variety of endpoints were monitored including clinical observations, clinical chemistry, differential blood analysis, and histopathology.

Reference substance (positive control)

no

Any other information on materials and methods incl. tables

Fish were exposed in dechlorinated Plymouth fresh water, with water changes every 12 hours. Fish were not fed 24 h prior to, or during the experiment in order to minimise the risk of the CNT absorbing to food or faecal material, and to help maintain water quality. Water samples were collected immediately before and after each water change for pH (YSI 63 pH meter), total ammonia (HI 95715, Hanna Instruments), dissolved oxygen (YSI 85 D.O. meter). There were no treatment differences in water quality between tanks (ANOVA, $P > 0.05$). Values were (means ± S.E.M., $n = 253$ -261 samples); total ammonia, 0.83 ± 0.18 mg/l. Photoperiod was 12 h light: 12 h dark.

Fish were graded into fifteen experimental glass aquaria (12 fish/tank), in a triplicate design (3

tanks/treatment), and allowed to rest for 24 h prior to the commencement of the experiment. Fish were exposed in triplicate to one of the following treatments for 10 days using a semi-static exposure regime (80% water change every 12 h with re-dosing after each change): control (freshwater only, no CNT or solvent), solvent control (0.15 mg/l sodium dodecyl sulphate, SDS), 0.1, 0.25 or 0.5 mg/l SWCNT. Fish were randomly sampled on day 0 (initial fish from the stock), day 4, and day 10 for haematology, plasma ions, tissue electrolytes, histopathology, and biochemistry. Behaviours were noted throughout the experiment, and ventilation rates (30 s visual count of opercular movements, in triplicate on each fish) were measured.

Results and discussions

Details on results

SWCNT exposure caused a dose-dependent rise in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), and mucus secretion with SWCNT precipitation on the gill mucus. No major haematological or blood disturbances were observed in terms of red and white blood cell counts, haematocrits, whole blood haemoglobin, and plasma Na^+ or K^+ . Tissue metal levels (Na^+ , K^+ , Ca^{2+} , Cu , Zn and Co) were generally unaffected. However some dose-dependent changes in brain and gill Zn or Cu were observed (but not tissue Ca^{2+}), that were also partly attributed to the solvent. SWCNT exposure caused statistically significant increases in $\text{Na}^+\text{K}^+\text{-ATPase}$ activity in the gills and intestine, but not in the brain. Thiobarbituric acid reactive substances (TBARS) showed dose-dependent and statistically significant decreases especially in the gill, brain and liver during SWCNT exposure compared to controls. SWCNT exposure caused statistically significant increases in the total glutathione levels in the gills (28%) and livers (18%), compared to the solvent control. Total glutathione in the brain and intestine remained stable in all treatments. Pathologies in the brain included possible aneurisms or swellings on the ventral surface of the cerebellum. Liver cells exposed to SWCNT showed condensed nuclear bodies (apoptotic bodies) and cells in abnormal nuclear division. Overt fatty change or wide spread lipidosis was absent in the liver. Fish ingested water containing SWCNT during exposure (presumably stress-induced drinking) which resulted in precipitated SWCNT in the gut lumen and intestinal pathology. Aggressive behaviour and fin nipping caused some mortalities at the end of the experiment, which may be associated with the gill irritation and brain injury, although the solvent may also partly contributed to aggression.

Any other information on results incl. tables

Overall the study concluded that SWCNTs are a respiratory toxicant in trout, the fish are able to manage oxidative stress and osmoregulatory disturbances, but other cellular pathologies raise concerns about cell cycle defects, neurotoxicity, and as yet unidentified blood borne factors that possibly mediate systemic pathologies. Although, some evidence of solvent control effects were apparent (for the majority of end points there was no solvent-effect), the SWCNT effect when present was statistically different from these controls, as is most easily explained by SWCNT exposure. Some subtle disturbances to individual trace elements, and further investigations of especially Zn and Cu were suggested. The absence of pathologies associated with lipid peroxidation in the liver and brain, and the reduction in TBARS in most tissues suggest that the fish were not suffering from overt oxidative stress. Examination of the brain and associated blood vessels raises new concerns about subtle neurotoxic or cardiovascular effects of SWCNTs that may alter fish behaviour (this has since been documented for other NPs). The cellular pathologies in the liver are indicative of genotoxicity or cell cycle defects, and raises the concern of carcinogenicity in longer term exposures. Several observations also suggests that SWCNT may be a

surface acting toxin on the gills of fish, and mediates a systemic effect via other inflammatory, cell cycle, or vasoactive factors in the blood. The vascular injury on the brain, liver cell pathologies close to blood vessels, but the histological absence of large polymerised CNT fibrous plaques inside any of the tissues examined support this notion. Further investigations of such factor in the blood are needed. One limitation of the study was that concentrations of SWCNT could not be measured inside the tissues, as methodology for such measurements did not exist in 2007, and this remains a technical problem for all studies. Some concerns are also raised about OECD tests incorporating only one solvent control and the importance of keeping the material: solvent ratio constant.

Endpoint study record: NRC SWCNT E3 medium Acute toxicity to fish by Canada.001

Administrative Data

Purpose flag supporting 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
study report		2010	SWCNT Toxicity to Fish-E3 medium						

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD Fish Embryo Toxicity Test(May 2006)	yes (modified)

GLP compliance

no

Test materials

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

no

Details on test material

10-20 nm diameter Single walled carbon nanotube bundles, 1.2-1.4 nm diameter and ~ 1-2 µm in length,
 Specific surface area: 190 m²/g,
 Surface chemistry (coating, Functionalized): none,
 Degree of purity: ~20 % SWCNTs, Carbonaceous impurities (amorphous carbon and carbon black) and
 metal catalyst particles (Ni and Y at 11% and 3% respectively),
 Method of production: Plasma method,
 Manufacturer: NRC,
 CAS number: 308068-56-6

Test organisms**Test organisms (species)**

Danio rerio

Details on test organisms

Danio rerio (zebrafish)/wildtype AB/Source, University of Alberta, 3 female:2 males
 Age at study initiation: 1-2 hours post fertilization

Study design**Test type**

static

Water media type

saltwater

Total exposure duration

96 h **Remarks** hours post fertilization

Post exposure observation period

96 h

Test conditions***Hardness***

E3 medium (5mM NaCl, 0.17mM KCl, 0.33mM CaCl₂•2H₂O, 0.33mM MgSO₄•7H₂O)

Test temperature

28.5°C

pH

7.2

Nominal and measured concentrations

Doses/concentration levels: 200 mg/L

Stock solutions preparation : 1g/L in deionized water

Details on test conditions

6 replicates/treatment or control

Exposure vessel type: Corning costar 6 well plates, 5mL/treatment

Lighting : 14h

light:10h dark

Frequency of treatment: Once, exposed within 1-2 hours of fertilization

Exposure period: 96hpf-120hpf

Results and discussions

Details on results

Biological observations: No effects on survival

Any other information on results incl. tables

Table showing cumulative SURVIVAL (%): (HA = with 10mg/L humic acid addition)

Summary survival (%)	24h		48h		72h		96h	
	average	std error	average	std error	average	std error	average	std error
CONT	88	1	88	1	88	1	88	1
HA+CONT	90	2	90	2	90	2	90	2
SWCNT 200	87	2	87	2	87	2	87	2
SWCNT 200 + HA	93	3	93	3	93	3	93	3
Summary hatch(%)	48h		72h		96h			
	average	std error	average	std error	average	std error		
CONT	27	5	98	2	100	0		
HA+CONT	8	3	100	0	100	0		
SWCNT 200	44	4	100	0	100	0		
SWCNT 200 + HA	27	12	99	1	100	0		

Endpoint study record: NRC pristine SWCNTs SIDS Fish acute toxicity by Canada.002

Administrative Data

Purpose flag supporting study

Study result type experimental result **Study period** 2010-2011

Reliability 4 (not assignable)

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	other guideline: OECD Fish Embryo Toxicity Test	yes (modified)

GLP compliance

no

Test materials

Details on test material

10-20 nm diameter Single walled carbon nanotube bundles,

Diameter, length: 1.2-1.4 nm diameter and ~ 1-2 µm in length,

Specific surface area: 190 m²/g,

Degree of purity: ~20 % SWCNTs, Carbonaceous impurities (amorphous carbon and carbon black) and metal catalyst particles (Ni and Y at 11% and 3% respectively)

Method of production: Plasma method, Manufacturer: NRC.

Test organisms

Test organisms (species)

Danio rerio

Details on test organisms

Danio rerio (zebrafish)/wildtype AB/University of Alberta 3 female:2 males

Age at study initiation: 1-2 hours post fertilization

Study design

Test type

static

Water media type

freshwater

Total exposure duration

96 h **Remarks** hours post fertilization

Post exposure observation period

96 h

Test conditions

Hardness

general water hardness 160 ppm, and carbonate water hardness 90 ppm

Test temperature

28.5°C

pH

7.57

Nominal and measured concentrations

Doses/concentration levels: 1, 10, 100 mg/L

Stock solutions: approx. 1g/L in deionized water

Details on test conditions

Exposure vessel type: Corning costar 6 well plates, 5mL/treatment 6 replicates/treatment or control

Exposure period: 96hpf-120hpf

Lighting : 14h light:10h dark

Results and discussions

Details on results

Biological observations: None

No significant mortality

Lowest test substance concentration causing 100% mortality: >100 mg/L

No effect on morphology or behaviour (spontaneous movement within the chorion at 48hpf, nor response to touch at 72hpf).

Endpoint study record: NRC SWCNTs dechlorinated tap water Fish acute toxicity by Canada.003

Administrative Data

Purpose flag supporting study

Study result type experimental result **Study period** 2011-2012

Reliability 4 (not assignable)

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD Fish Embryo Toxicity Test(May 2006)	

GLP compliance

no

Test materials

Test material identity

Identifier	Identity
other:	NRC-SIMS SWCNT

Details on test material

Pristine or un-functionalized SWCNTs; 10-20 nm bundles,

Diameter, length: 1.2-1.4 nm diameter and ~ 1-2 µm in length,

Specific surface area: 190 m²/g,

Degree of purity: ~20 % SWCNTs

Carbonaceous impurities (amorphous carbon and carbon black) and metal catalyst particles (Ni and Y at 11% and 3% respectively)

Method of production: Plasma method, Manufacturer: NRC. COOH 30 SWCNTs Carboxylic acid functionalized SWCNTs prepared by oxidation in a HNO₃/H₂SO₄ at 30°C of SWCNTs manufactured by NRC. COOH 40 SWCNTs Carboxylic acid functionalized SWCNTs prepared by oxidation in a HNO₃/H₂SO₄ at 40°C of SWCNTs manufactured by NRCCOOH 50 SWCNTs Carboxylic acid functionalized SWCNTs prepared by oxidation in a HNO₃/H₂SO₄ at 50°C SWCNTs manufactured by NRC

Test organisms

Test organisms (species)

Danio rerio

Details on test organisms

Danio rerio (zebrafish) / wild type AB / University of Alberta, 1 female : 2 males

Age at study initiation: 1-2 hours post fertilization

Study design

Test type

static

Water media type

freshwater

Total exposure duration

72 h

Post exposure observation period

0 hours

Test conditions***Hardness***

general water hardness 160 ppm, and carbonate water hardness 90 ppm

Test temperature

28.5°C

pH

7.57

Nominal and measured concentrations

1, 10, 50, 100, 200mg/L

Stock solutions: 1g/L SWCNTs in deionized water

Dilution water source: de-chlorinated Edmonton tap water from aquatics' facility at University of Alberta

Details on test conditions

Frequency of treatment: Once

Exposure vessel type: Corning Costar 6 well plates, 1 well per treatment

Lighting: 14h light, 10h dark

Water chemistry in test (D.O., pH) in the control and at least one concentration where effects were observed: control pH = 7.57, stock solution pH = 6.93, delay in hatch at 28, 52 and 56 hours post fertilization.

Analytical monitoring: death, hatch and morphological impairment at 24, 48 and 72 hours post fertilization

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
	LC100	> 200 mg/L				

Details on results

No significant mortality compared to dechlorinated tap water control.

Mortality of controls: $\leq 20\%$

No effect on morphology or hatch.

Any observations, such as precipitation that might cause a difference between measured and nominal values: Sedimentation over exposure period.

Applicant's summary and conclusion**Conclusions**

Preliminary results show SWCNTs are not acutely toxic.

6.1.2 Long-term toxicity to fish**Endpoint study record: Nikkisso SWCNT Long-term toxicity to fish.001****Administrative Data**

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. This study is conducted under GLP using OECD Test Guideline

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	Prolonged Toxicity test of Nikkisso SWCNT using Oryzias latipes		Mitsubishi Chemical Medience Corp.	A100698			

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 SWCNT

Vehicle

yes

Details on test solutions

Concentration level:

Test substance; 0.10, 0.32, 1.0, 3.2, 10 mg/L,

Dispersing agent (Tween 80); 100 mg/L

Test organisms**Test organisms (species)**

Oryzias latipes

Details on test organisms

length:1.91-2.43cm(av.2.08cm, n=10

)weight:0.057-0.099g(av.0.073g, n=10)

Study design

Test type

semi-static

Water media type

freshwater

Total exposure duration

14 d

Test conditions

Hardness

53 mg(CaCO₃)/L

Test temperature

24+/-1 C

pH

7.5

Dissolved oxygen

5.5-8.4mg/L

Nominal and measured concentrations

Concentration level:

Test substance; 0.10, 0.32, 1.0, 3.2, 10 mg/L,

Control: Control; no test substance and dispersing agent

Vehicle Control; dispersing agent only

Dispersing agent(Tween 80); 100 mg/L

Details on test conditions

Semi-static test with moderate aeration,

Change of solution: 3 times/week

Exposure duration: 14 days,Light: 16 hours photoperiod daily,

Test Item: Lethal effects and effects other than lethal effects

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	ca. 10 mg/L				
14 d	LOEC	> 10 mg/L				
14 d	EC50	> 10 mg/L				

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	10	10	9	9	9	10
	Death	0	0	0	0	0	0	0	0	0	0	0	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	
3.2mg/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	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	

Observation of Abnormal Symptoms

Test group	Number of survival and dead Fish					
	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14
Control	N	N	N	N	N	N
Sol. Cont.	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
3.2mg/L	N	N	N	N	N	N
10 mg/L	N	N	N	N	N	N

N: No abnormal symptoms were observed.

Observation of Feeding Activity

Test group	Feeding Activity					
	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14
Control	N	N	N	N	N	N
Sol. Cont.	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

3.2mg/L	N	N	N	N	N	N
10 mg/L	N	N	N	N	N	N

N: No abnormal symptoms were observed.

Wet Body Weight of Survival Fish at the End of Exposure

	Control	Sol. Cont.	0.10 mg/L	0.32 mg/L	1.0 mg/L	3.2 mg/L	10 mg/L
Wet body weight (mg)	102	148	142	153	143	121	107
	126	180	110	114	118	89	176
	118	141	95	135	138	102	108
	100	100	142	133	148	141	116
	65	102	113	118	167	90	187
	78	118	64	169	108	145	131
	156	117	115	162	158	129	97
	87	140	119	96	136	117	148
	114	85	121	97	143	130	95
	-	93	90	93	195	84	128
n	9	10	10	10	10	10	10
Total Mean	105	122	111	127	145	115	129
Total S. D.	27.3	29.5	23.6	28.0	24.5	22.3	32.0
Total C.V.	26.0	24.1	21.2	22.0	16.9	19.4	24.7

n : Total number of fish measured.

Total Body Length of Survival Fish at the End of Exposure

	Control	Sol. Cont.	0.10 mg/L	0.32 mg/L	1.0 mg/L	3.2 mg/L	10 mg/L
Wet body weight (mg)	23.3	23.5	24.1	23.8	21.8	21.5	20.3
	25.0	25.6	23.4	23.3	21.1	19.8	23.7
	24.0	25.0	22.4	23.7	24.1	23.3	20.0
	23.7	21.9	23.8	24.4	22.7	22.8	23.4
	20.9	21.1	23.5	23.1	23.9	20.7	26.3
	21.0	23.4	22.0	25.9	21.7	24.7	22.1
	25.7	23.4	23.2	26.1	24.0	24.9	21.7
	22.9	25.4	25.2	21.8	23.6	23.4	25.1
	24.0	21.2	23.9	21.2	22.2	22.5	19.6
	-	20.7	20.7	22.4	26.3	20.3	21.3

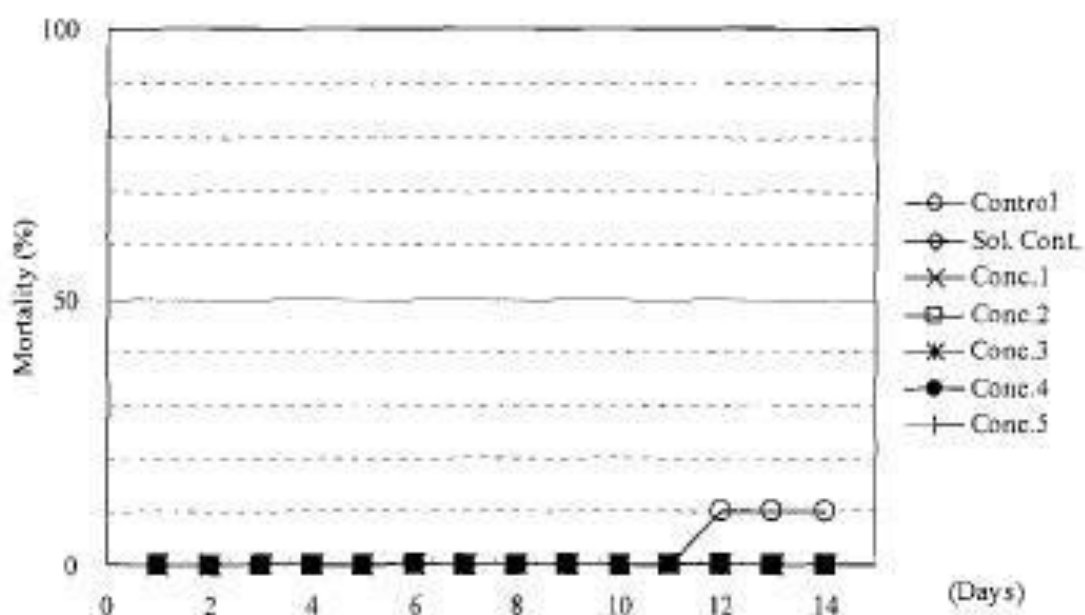
n	9	10	10	10	10	10	10
Total Mean	23.4	23.1	23.2	23.6	23.1	22.4	22.4
Total S. D.	1.62	1.83	1.25	1.60	1.55	1.78	2.24
Total C.V.	6.9	7.9	5.4	6.8	6.7	7.9	10.0

n : Total number of fish measured.

Overall remarks, attachments

Illustration (picture/graph)

Figure 1 Cumulative Mortality during Exposure



Endpoint study record: Super Growth SWCNT Long-term toxicity to fish.001**Administrative Data**

other:OECD/WPMN

Purpose flag key study**Study result type** experimental result**Reliability** 1 (reliable without restriction)**Rationale for reliability incl.** This study is conducted under GLP using OECD Test Guideline**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	Prolonged Toxicity test of Super GRowth SWCNT using Oryzias latipes		Mitsubishi Chemical Medience Corp.	A110430			

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:	Super Growth SWCNT

Vehicle

yes

Details on test solutions

Concentration level:

Test substance; 0.10, 0.32, 1.0, 3.2, 10 mg/L,

Dispersing agent(Tween 80); 100 mg/L

Test organisms

Test organisms (species)

Oryzias latipes

Details on test organisms

length:2.20-2.68cm (av.2.44cm, n=10)

weight:0.092-0.155g(av.0.122g, n=10)

Study design

Test type

semi-static

Water media type

freshwater

Total exposure duration

14 d

Test conditions

Hardness

51 mg(CaCO₃)/L

Test temperature

24+/-2 C

pH

7.8

Dissolved oxygen

>60% of saturated dissolved oxygen

Nominal and measured concentrations

Concentration level:

Test substance; 0.10, 0.32, 1.0, 3.2, 10 mg/L,

Control: Control; no test substance and dispersing agent

Vehicle Control; dispersing agent only

Dispersing agent(Tween 80); 100 mg/L

Details on test conditions

Semi-static test with moderate aeration,

Change of solution: 3 times/weekExposure duration: 14 days,

Light: 16 hours photoperiod daily,

Test Item: Lethal effects and effects other than lethal effects

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	ca. 10 mg/L				
14 d	LOEC	> 10 mg/L				
14 d	EC50	> 10 mg/L				

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	10	10	10	10	10	0
	Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
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	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	

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

N:(No abnormal response)

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

N:(No abnormal response)

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)	200	200	154	135	118	140	163
	104	121	136	142	140	163	155
	178	148	164	241	237	143	158
	170	161	221	107	158	244	162
	150	171	164	239	151	142	135
	107	133	138	133	107	146	143
	100	199	212	215	142	115	143
	157	138	183	209	158	201	168
	178	92	133	160	132	145	151
	279	195	143	162	161	137	242
N	10	10	10	10	10	10	10
Total Mean	162.3	155.8	164.8	174.3	150.4	157.6	162.0
Total S. D.	53.9	36.2	31.4	47.9	35.2	37.5	30.0
Total C.V.	33.2	23.2	19.1	27.5	23.4	23.8	18.5

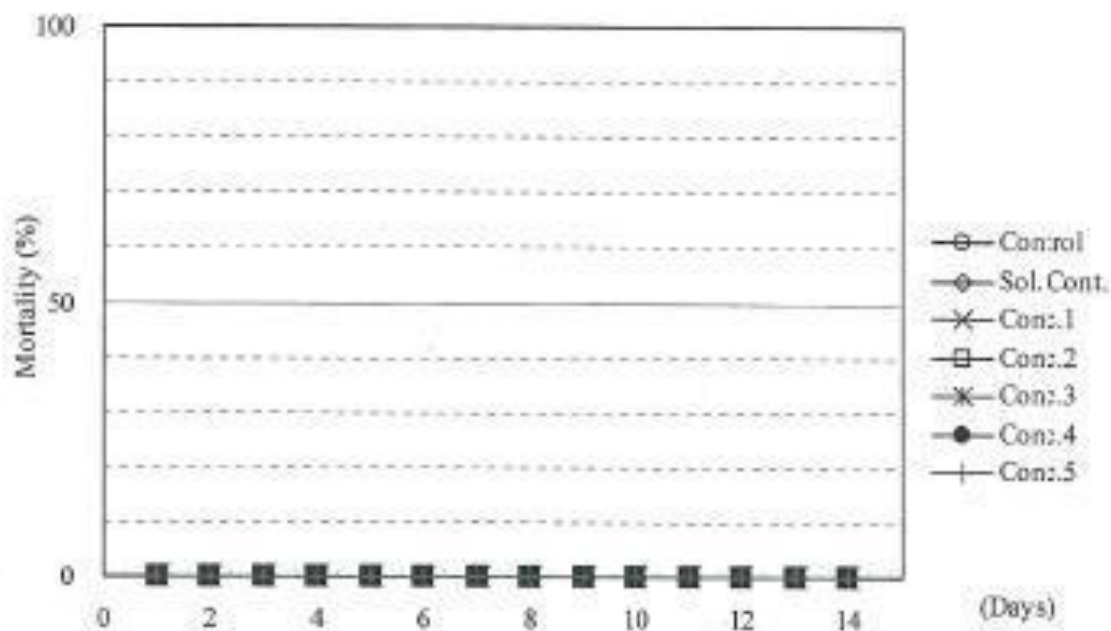
Total Body Length of Survival Fish at the End of Exposure

	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)	29.5	24.5	25.7	22.6	22.7	25.2	25.7
	22.6	23.0	24.7	26.2	24.2	25.9	24.5
	26.7	25.1	28.0	29.8	30.0	25.3	27.2
	26.3	25.6	28.9	22.8	24.4	29.3	26.7
	24.6	27.3	26.7	28.6	24.8	24.5	24.3
	21.6	24.9	24.9	24.4	21.8	24.5	24.0
	22.2	28.2	28.1	29.2	24.0	22.7	23.4
	26.4	26.0	26.9	28.9	24.9	27.9	25.2
	27.4	21.2	24.7	27.4	26.1	25.8	26.8
	29.4	26.8	25.7	27.5	25.8	24.6	29.8
N	10	10	10	10	10	10	10
Total Mean	25.7	25.3	26.4	26.7	24.9	25.6	25.8
Total S. D.	2.8	2.1	1.5	2.6	2.2	1.9	1.9
Total C.V.	10.9	8.3	5.7	9.7	8.8	7.4	7.4

Overall remarks, attachments

Illustration (picture/graph)

Figure 1 Cumulative Mortality during Exposure



Endpoint study record: Cheap Tubes SWCNT Long-term dietary toxicity to fish.001**Administrative Data****Purpose flag** supporting study**Study result type** experimental result**Study period** 2010**Reliability** 1 (reliable without restriction)

Rationale for reliability incl. deficiencies This study used the well accepted approach used in industry for a triplicated nutrition trial design with a detailed nutritionally competent diet formulation, and the OECD has not yet developed protocols to this technical level with respect to dietary toxicity in fishes. However, like aqueous tests water quality measurements were made, and food intake was measured to confirm ingested dose. Numerous sub-lethal end points were included. There are no OECD protocols for many of the physiological end points, but the exposure itself was to GLP standards in an academic laboratory.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Fraser et al.	2011	Dietary toxicity of single-walled carbon nanotubes and fullerenes (C60) in rainbow trout (Oncorhynchus mykiss)	Nanotoxicology, 5 (1), 98-108(2011)					

Materials and methods**Life stage / endpoint studied**

juvenile fish: growth

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: Cheap Tubes	

Details on test material

Single Walled Carbon Nanotubes obtained from Cheap Tubes Inc. (Vermont, USA), and had (manufacturer's information) 1.1 nm mean outside diameter, 5–3 µm length, and the powder was a minimum of 96.3% carbon, (maximum impurities were: Al 0.08, Cl 0.41, Co 2.91, and S 0.29%).

Details on test solutions

It was supplied as a dry powder. This was then made into a stock dispersion. However, analysis of the stock dispersion for metals revealed these impurities were below detection (< 0.1 µg/l by ICP-OES). A stock solution (1 litre) were prepared by dispersing 5.0 g/l SWCNT in 20 g/l SDS and then sonicating for 2.5 h in a bath-type sonicator (35 kHz frequency, Fisherbrand FB 11010, Germany). This container was then stirred for 3.5-5.5 h to mix without causing foaming of the detergent. The nanomaterials were then added to the dry animal feed pellets by spraying during mixing of the food, to ensure even dispersion of the NMs among food pellets (% of dry weight from manufacturer's guidelines for Trout Excel 18: lipid 18; protein 50; ash 8; fibre 1; phosphorus 1.2), the dietary marker yttrium oxide was added (15 g/l) to each stock solution. The diet was then sealed with a top coat of 3.3% bovine gelatine to prevent leaching.

Test organisms**Test organisms (species)**

other: Juvenile rainbow trout

Details on test organisms

Normal juvenile rainbow trout, *Oncorhynchus mykiss*, weigh at the start of the experiment 17.7 g ± 0.6; mean ± SEM).

Study design**Test type**

static

Water media type

freshwater

Total exposure duration

6 wk

Post exposure observation period

2 weeks

Test conditions***pH***

6.72 ± 0.02

Dissolved oxygen

percent oxygen saturation, 89.8 ± 0.37%

Salinity

The electrolyte composition of system water was 0.3, 0.04, 0.08 and 0.4 mM for Ca, K, Mg, and Na, respectively.

Details on test conditions

Actual ingested dose (food eaten) was monitored in each tank (9 tanks, 45 fish/tank). Fish were only fed to satiation so there was minimal food wastage and any uneaten pellets were immediately removed from the tanks. Fish were held in recirculating Plymouth fresh water (aquarium system with a biofilter etc). Water samples were collected twice daily (prior to feeding), from the tank outflows, and measurements taken of water pH, temperature, and dissolved oxygen (HACH HQ40d multi reader). Total ammonia nitrogen and nitrite were measured daily, while nitrate was monitored once every two days (HACH LANGE GMBH LCK 304, 341, and 340 kits, respectively, and read on HACH LANGE GmbH DR 2800 spectrophotometer). Values were (mean ± SEM, n in brackets), pH, 6.72 ± 0.02 (86); temperature, 15.7 ± 0.05 °C (85); percent oxygen saturation, 89.8 ± 0.37% (85); ammonia < 0.01 mg/l (42); nitrite, 0.44 ± 0.03 mg/l (47); nitrate, 3.37 ± 0.31 mg/l (22). The electrolyte composition of system water was 0.3, 0.04, 0.08 and 0.4 mM for Ca, K, Mg, and Na, respectively.

Any other information on materials and methods incl. tables**Details of Exposure Duration;**

During the experiment fish were exposed (six weeks) to either control (no CNs), SWCNTs (500 mg/kg food), or C60 (500 mg/kg food); and exposure was followed by a two-week recovery period in which all fish were fed the control trout food. Fish were offered food twice per day (10:00 and 16:00 h), and the total amount of food offered per day was 2.8% of their body weight.

Details of Protocol;

Triplicated fish nutrition design (3 treatments, 9 tanks, 3 tanks/treatment each containing 45 fish at the start of the experiment). Fish were fed 2.8 % body weight of one of the appropriate diets above for 6 weeks, followed by a further 2 weeks on the control diet. Fish were sampled for assessment of

haematology, plasma ions, tissue electrolytes, histopathology, and biochemistry. In addition, nutritional performance and growth were monitored and recorded. Proximate composition analysis of whole fish (ash, lipid, protein, and moisture) was carried out at weeks 0 and 6 (6 fish per treatment, $n = 6$ for initial fish). Feeding behaviours were also noted.

Results and discussions

Results with reference substance (positive control)

The results were mainly negative indicating limited effects of dietary SWCNT at the doses and duration used. Fish growth and food intake was normal, with the fish readily eating all the diets. There were no treatment differences in the proximate composition of the carcasses or in their trace element contents at the end of the exposure confirming that nutrition of the animals was not compromised. Exposure to the SWCNT had no statistically significant effect on plasma Na^+ , K^+ , plasma protein or glucose levels. $\text{Na}^+\text{K}^+\text{-ATPase}$ activity in the gill, intestine, and brain were not affected by dietary exposure to the materials. A significant elevation in brain TBARS (an indication of lipid peroxidation) was observed in fish exposed to SWCNTs (16.2 ± 1.38 nmol/mg protein) compared to the control (9.11 ± 0.81 nmol/mg protein), but without depletion of brain glutathione levels (chemical anti-oxidant). The histological examinations of gills, intestines and brains showed only a background incidence of lesions or very minor effects that were not easily attributed to the nano treatments. However, macrophage aggregates (inflammation) were observed in 2 fish from the SWCNT treatment.

Overall remarks, attachments

Remarks on results including tables and figures

This study is one of the first to address the dietary exposure hazard from SWCNTs and C60 in fish. There was no shape-effect observed (no differences between SWCNT and C60 treatments at equivalent ingested doses). Apart for some transient changes in TBARS and some minor pathologies (perhaps with the exception of some livers), there were no effects on growth, haematology, or tissue Electrolytes; suggesting the hazard from ingesting 500 mg/kg SWCNTs or C60 appears to be relatively low compared to other dietary contaminants of concern (e.g., toxic metals, see Handy et al. 2005 in: Toxicity of Dietborne Metals to Aquatic Organisms, SETAC press). From a regulatory perspective the dietary exposure is less hazardous than other substances examined in the Plymouth lab in similar conditions (less toxic than Hg, Cu for example). However, novel toxic effects from the materials on endpoints not measured remain a possibility. The absence of effects cannot be explained to no exposure, as food was ingested. In the absence of detection methods for tissues the internalisation of the materials to the body organs could not be measured, but to put this in context with substances like metals which are readily absorbed (uptake of a few % of the dose), it may only be expected that a few mg are internalised in the exposure leading to only low $\mu\text{g/g}$ amounts in the tissues. The only other dietary study (Ramsden et al., 2009 Ecotoxicology: 18, 939-951 on TiO_2) caused more effects, suggesting that SWCNT may be less toxic via the oral route than P25 TiO_2 .

6.1.3 Short-term toxicity to aquatic invertebrates

Endpoint study record: *Nikkiso SWCNT Short-term toxicity to aquatic invertebrates.001*

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted under GLP 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	48 hr Acute Immobilisation test of Nikkiso SWCNT using Daphnis magna		CERI	95402			

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 SWCNT

Vehicle

yes

Details on test solutions

Test substance: 10, 4.5, 2.1, 0.94, 0.42 mg/L
dispersing agent(HCO-40): 100mg/L

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

Obtained from University of Sheffield, acclimated at laboratory and used larva within 24 hr old.

Study design

Test type

static

Water media type

freshwater

Total exposure duration

48 h

Remarks No aeration, No change of solution, Light: 16 hours photoperiod daily, Feed: none

Test conditions***Hardness***27mg/L(as CaCO₃)***Test temperature***

20+/-1 C

pH

7.3

Dissolved oxygen

8.8-8.9 mg/L

Nominal and measured concentrations

10, 4.5, 2.1, 0.94, 0.42 mg/L

Details on test conditions

Control: Control; no test substance and dispersing agent

Vehicle Control; dispersing agent only,

Dispersing agent was HCO-40

Test Item: immobility, abnormal behaviour or appearance

Number of species per sex per dose: Five daphnias in each test (n=4), control (n=4) or vehicle control (n=4) solution

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				

Details on results

48 hr 0% immobility maximum concentration ;4.5mg/L48 hr 100% immobility minimum concentration>10mg/L

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	
0.42	A	0	0	0	0
	B	0		0	
	C	0		0	
	D	0		0	
0.94	A	0	0	0	0
	B	0		0	
	C	0		0	
	D	0		0	
2.1	A	0	0	0	5
	B	0		20	
	C	0		0	
	D	0		0	
4.5	A	0	0	0	0

	B	0		0	
	C	0		0	
	D	0		0	
10	A	0	0	40	25
	B	0		0	
	C	0		20	
	D	0		40	

Observed abnormal response

Nominal concentration (mg/L)	Immobility (%)			
	24 hours		48 hours	
	Immobilization	Other symptom	Immobilization	Other symptom
Control	-	-	-	-
Vehicle control			-	-
0.42	-	-	-	ADS(6)
0.94	-	-	-	ADS(7)
2.1	-	ADS(3)	IM(1)	RA(1) ADS(7)
4.5	-	ADS(5)	-	RA(6) ADS(7)
10	-	ADS(6)	IM(5)	RA(6) ADS(6)

-(No abnormal response)

Abbreviation of symptoms

1M : Immobilization

RA : Reduced activity

ADS : Adhesion of the substance

Endpoint study record: Super Growth SWCNT Short-term toxicity to aquatic invertebrates.001**Administrative Data**

other:OECD/WPMN

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

The test was conducted under GLP 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	48 hr Acute Immobilisation test of Super Growth SWCNT using Daphnis magna		CERI	95406			

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:	Supergrowth SWCNT

Vehicle

yes

Details on test solutions

Test substance: 10, 4.5, 2.1, 0.94, 0.42 mg/L
dispersing agent (HCO-40): 100 mg/L

Test organisms**Test organisms (species)**

Daphnia magna

Details on test organisms

Obtained from University of Sheffield, acclimated at laboratory and used larva within 24 hr old.

Study design**Test type**

static

Water media type

freshwater

Total exposure duration

48 h

Remarks No aeration, No change of solution, Light: 16 hours photoperiod daily, Feed: none

Test conditions

Hardness

27mg/L(as CaCO₃)

Test temperature

20+/-1 C

pH

7.3

Dissolved oxygen

8.7-8.9 mg/L

Nominal and measured concentrations

10, 4.5, 2.1, 0.94, 0.42 mg/L

Details on test conditions

Control: Control; no test substance and dispersing agent

Vehicle Control; dispersing agent only,

Dispersing agent was HCO-40

Test Item: immobility, abnormal behaviour or appearance

Number of species per sex per dose: Five daphnias in each test (n=4), control (n=4) or vehicle control (n=4) solution

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				

Details on results

48 hr 0% immobility maximum concentration ;2.1mg/L
 48 hr 100% immobility minimum concentration>10mg/L

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	
0.42	A	20	5	20	5
	B	0		0	
	C	0		0	
	D	0		0	
0.94	A	0	0	0	0

	B	0		0	
	C	0		0	
	D	0		0	
2.1	A	0	0	0	0
	B	0		0	
	C	0		0	
	D	0		0	
4.5	A	0	0	0	10
	B	0		20	
	C	0		0	
	D	0		20	
10	A	0	0	0	5
	B	0		0	
	C	0		20	
	D	0		0	

Observed abnormal response

Nominal concentration (mg/L)	Immobility (%)			
	24 hours		48 hours	
	Immobilization	Other symptom	Immobilization	Other symptom
Control	-	-	-	-
Vehicle control	-	-	-	-
0.42	IM(1)	-	IM(1) LETH(1)	ADS(1)
0.94	-	-	-	RA(1) ADS(2)
2.1	-	-	-	RA(2) ADS(4)

4.5	-	-	IM(2)	RA(2) ADS(3)
10	-	-	IM(1) LETH(1)	RA(1)

-(No abnormal response)

Abbreviation of symptoms

1M : Immobilization

LETH: Lethargic

RA : Reduced activity

ADS : Adhesion of the substance

Endpoint study record: Shenzhen Nanotech Port SWCNT Short-term toxicity to aquatic invertebrates.001

Administrative Data

Purpose flag supporting 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	Zhu et al.	2009	Acute toxicities of six manufactured nanomaterial suspensions to Daphnia magna.	Nanopart. Res, 11:67-75					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	yes modifications in accordance with the OECD guidance document for difficult to test substances and mixtures(OECD 2000,2004)

Test materials**Test material identity**

Identifier	Identity
other: Shenzhen Nanotech Port	

Details on test material

The SWCNTs were produced by Shenzhen Nanotech Port Co., Ltd. and had the following information supplied from the manufacturer: diameter < 2 nm, length 5-15 um, purity > 60-90%.

Test organisms**Test organisms (species)**

Daphnia magna

Test conditions***Details on test conditions***

A stock solution was prepared in reconstituted water using vigorous shaking at room temperature (specifics not provided) to a final nominal concentration of 1,000 mg/L; dilutions of the stock were used for the 48-hour exposures of daphnids (0.1, 0.5, 1, 5, 10, 50, and 100 mg/L). Beakers containing test organisms were shaken constantly at 140 rpm, and daphnids were not fed during the test.

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	<= 1.306 mg/L				95%CI 0.821-1.994
48 h	LC50	<= 2.425 mg/L				95%CI 1.639-3.550

Overall remarks, attachments

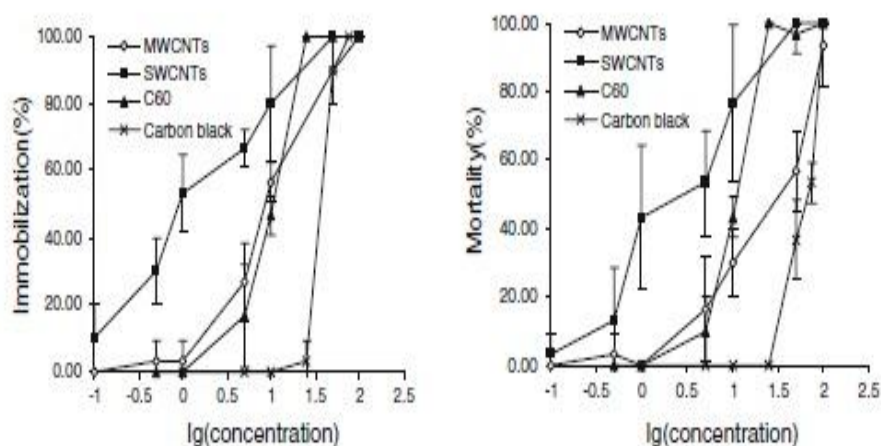
Remarks on results including tables and figures

A dose-dependent increase in toxicity was seen with increasing nominal SWCNT concentrations, with both immobilization and mortality assessed.

Large amounts of SWCNTs were found in the gut of the daphnids, and SWCNTs were found to be more toxic than MWCNTs

Illustration (picture/graph)

Fig. 1 Effects of suspensions of six NPs and their bulk counterparts on the immobilization and mortality of *D. magna*. Error bars represent one standard deviation of the mean



Endpoint study record: Shenzhen Nanotech Port SWCNT Short-term toxicity to aquatic invertebrates.002

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The published results were generated, analyzed, and reported according to the generally accepted scientific procedure and rigor.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Mwangi et al.	2012	Toxicity of carbon nanotubes to freshwater aquatic invertebrates	Env. Tox & Chem, 31:1823-1830					

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	other guideline: ASTM standard test protocols E1706-05 and E729-96	

Principles of method if other than guideline

The testing follows the ASTM guidelines when it is applicable. American Society for Testing and Materials (ASTM). 2006a. ASTM International standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates (ASTM E1706-05). Annual Book of ASTM Standards Volume 11.06, West Conshohocken, PA, pp 916-1023. American Society for Testing and Materials (ASTM). 2006b. Standard guide for conducting laboratory toxicity tests with freshwater mussels (ASTM E2455-06). In Annual Book of ASTM Standards, Vol. 11.06. West Conshohocken, PA, pp 1393-1444.

Test materials**Test material identity**

Identifier	Identity
other: Shenzhen Nanotech Port	

Details on test material

SWCNTs from Shenzhen Nanotech Port, China (with >90% dry weight purity, 2-nanometer average diameters, 5 to 15 micrometr average lengths, >400 m²/g specific surface area, <2% dry weight ash, and <5% dry weight amorphous carbon)

Details on test solutions

Two SWCNT stock solutions were prepared: one with sonication (2 min at 65 W) and stirring, and the

other with only stirring; no other dispersion agents were used.

Test organisms

Test organisms (species)

other: amphipods, midges and oligochaetes

Details on test organisms

Amphipod (*Hyalella azteca*), the midge (*Chironomus dilutus*), the mussel (*Villosa iris*), and the oligochaete (*Lumbriculus variegatus*)

Study design

Test type

static

Test conditions

Details on test conditions

Sediment-dwelling invertebrates in 14-day tests adapted from ASTM standard test protocols E1706-05 and E729-96, and each 200 ml volume of test solution containing the organisms and CNTs also contained 5 ml of fine sand. A single concentration of 1,000 mg/L SWCNTs was used, and only nominal concentrations were reported for the static tests (run with feeding, and replenishment of non-laden CNT water only).

Overall remarks, attachments

Remarks on results including tables and figures

Significant differences in body weight and survival were noted for amphipods, midges and oligochaetes relative to the plain water control; sonicated SWCNTs had significantly higher adverse effects than unsonicated SWCNTs.

Applicant's summary and conclusion

Conclusions

Mwangi et al (2012) examined the effects of SWCNTs on sediment-dwelling invertebrates in 14-day tests adapted from ASTM standard test protocols E1706-05 and E729-96, and each 200ml volume of test solution containing the organisms and CNTs also contained 5 ml of fine sand. The SWCNTs were produced by Shenshen Nanotech Port Co., Ltd. and had the following information supplied from the manufacturer: 2 nm average diameter, average length 5-15 μ , $\geq$ 400 m²/g specific surface area, $\leq$ 0.2% dry weight total impurities, $\geq$ 90% dry weight purity. A single concentration of 1,000 mg/L was used,

and only nominal concentrations were reported for the static tests (run with feeding, and replenishment of non-laden CNT water only). Two SWCNT stock solutions were prepared: one with sonication (2 min at 65 W) and stirring, and the other with only stirring; no other dispersion agents were used. Significant differences in body weight and survival were noted for amphipods, midges and oligochaetes relative to the plain water control; sonicated SWCNTs had significantly higher adverse American Society for Testing and Materials (ASTM).

Endpoint study record: Arc-discharge produced SWCNT Short-term toxicity to aquatic invertebrates.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Meets generally accepted scientific standards, well documented and acceptable for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Templeton et al.	2006	Life-cycle effects of single-walled carbon nanotubes (SWNTs) on an Estuarine Meiobenthic copepod	Env. Sci & Technol., 10-1021					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: ASTM E-2317-04	

Test materials

Test material identity

Identifier	Identity
other:	

Details on test material

Chemically purified arc-discharge produced SWCNTs. SWCNTs were produced by a bench-scale arc discharge method, and purified by reflux in 3.3 N nitric acid (which removed metallic and carbonaceous contaminants). This chemical process oxidized the SWCNTs at their termini and defect locations, and incorporated hydroxyl and carboxylic acid functional groups (which increased SWCNT water solubility)

Test organisms

Test organisms (species)

other: estuarine meiobenthic copepod *Amphiascus tenuiremis*

Test conditions

Details on test conditions

Tests were conducted according to a standardized ASTM protocol (ASTM E-2317-04), in 200 µl microplate assays where media were replaced every 4 days. Full life-cycle studies on survival/developmental/reproductive endpoints with the estuarine meiobenthic copepod *Amphiascus tenuiremis* exposed to chemically purified arc-discharge produced SWCNTs at sedimentary concentrations of 0, 0.58, 0.97, or 1.5, or 10 mg/L.

Results and discussions

Results with reference substance

SWCNTs with metallic contaminants showed significant toxicity at 10 mg/L relative to survival, developmental success, development rate, and fertilization success. Purified SWCNTs showed no effects on mortality, development and reproduction across exposures. Interestingly, an extract of the unpurified SWCNTs (which contained small fluorescent nanocarbon byproducts and no metallic impurities) resulted

in significantly increased life cycle mortality at 10 mg/L, and caused significant reduction in life cycle molding success even at the lowest concentration tested (0.58 mg/L). This fluorescent fraction was fully soluble in aqueous test solutions; its particles were less than 18 nm in length, with equal widths and heights near 1 nm.

6.1.4 Long-term toxicity to aquatic invertebrates

Endpoint study record: Nikkiso SWCNT 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 The test was conducted under GLP 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	TASC (Technology Research Association for Single Wall Carbon Nanotubes)	2011	Daphnia magna Reproduction Test		Mitsubishi Chemical Medience Corp.	A100699			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 211 (Daphnia magna Reproduction Test)	no

GLP compliance

yes (incl. certificate)

Test materials**Test material identity**

Identifier	Identity
other:	Nikkiso SWCNT

Details on test material

Lot No. SW1884

Details on test solutions

1000mg/L stock solution was made by crushing freeze dried samle and dispersing with Tween 80
 10000mg/L test water.0.0030, 0.0095, 0.030, 0.095, 0.30 mg/L solution was prepared by diluting stock
 solution with test water.Concentrations were not analysed. Elendt M4 medium recommended by OECD
 TG 211 was used as dilution water.

Test organisms**Test organisms (species)**

Daphnia magna

Details on test organisms

Source: National Institute for Environmental Studies

Study design**Test type**

semi-static

Water media type

freshwater

Total exposure duration

21 d

Test conditions

Hardness

<250 mg CaCO₃ / L

Test temperature

20± 1 °C

pH

6.0-9.0 (variation < 1. 5)

Dissolved oxygen

>3 mg/L, no aeration

Nominal and measured concentrations

0.0030, 0.0095, 0.030, 0.095, 0.30 mg/L (nominal)

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

Reference substance (positive control)

no

Results and discussions

Effect concentrations

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

Any other information on results incl. tables

Table8. 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	8.2	18.3	18.3	18.3	51.1	51.1	67.0	82.6	88.3	88.3	104.0	107.1	107.1	129.8
Solvent control	--	0.0	16.1	17.6	17.6	17.6	52.6	52.6	80.4	91.3	91.3	91.3	111.4	111.4	111.4	143.4
0.10 mg/L	0.0030	0.0	17.3	17.3	17.4	17.4	52.3	52.3	88.9	88.9	88.9	88.9	107.1	107.1	107.1	142.0
0.32 mg/L	0.0095	0.0	16.0	16.0	20.0	20.0	49.0	49.0	85.0	89.0	89.0	89.0	114.0	114.0	115.0	149.0

L		0	8	8	4	4	0	0	4	3	3	3	5	5	2	2
1.0 mg/ L	0.03 0	0 . 0	1 2. 8	1 4. 4	1 4. 4	1 4. 4	4 6. 6	4 6. 6	6 7. 1	8 5. 3	8 5. 3	8 5. 3	10 8. 8	10 8. 8	10 8. 8	13 8. 4
3.2 mg/ L	0.09 5	0 . 0	1 4. 1	1 4. 1	1 4. 1	1 4. 1	4 8. 3	4 8. 3	8 2. 1	8 6. 1	8 6. 1	8 6. 1	10 9. 9	10 9. 9	10 9. 9	14 6. 6
10 mg/ L	0.30	0 . 0	7. 3	1 6. 6	1 6. 6	1 6. 6	4 7. 1	4 7. 1	6 5. 1	8 0. 4	8 5. 1	8 5. 1	10 5. 3	10 9. 6	10 9. 6	13 6. 3

Table9. Reproduction Rate, Inhibition Rate and Results of Statistical Comparison

Test Group	Nominal Conc. (mg/L)	Cumulative Number of Offsprings										Mean	S.D.	Reprod. Rate (%)	Inhib. Rate	Signific.diff.
		Vessel No.														
		1	2	3	4	5	6	7	8	9	10					
Control	--	D	102	143	114	154	140	105	146	135	129	129.8	18.7	--	--	--
Solvent control	--	D	142	148	134	154	142	D	145	139	D	143.4	6.4	--	--	--
1	0.030	D	D	152	156	156	D	121	140	132	137	142.0	13.3	99.0	1.0	-
2	0.095	151	159	140	154	135	147	152	152	142	160	149.2	8.1	104.0	-4.0	-
3	0.30	135	D	133	130	145	D	138	165	105	156	138.4	18.1	96.5	3.5	-

4	0.0 95	1 3 5	1 4 4	1 4 5	1 5 5	D	D	1 3 9	1 5 6	D	1 5 2	14 6. 6	8. 1	102 .2	- 2.2	-
5	0.3 0	D	1 2 2	1 1 5	1 2 9	1 4 5	1 3 7	D	1 4 5	1 4 8	1 4 9	13 6. 3	1 2. 9	95. 0	5.0	-

Endpoint study record: Super Growth SWCNT 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 The test was conducted under GLP 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	TASC (Technology Research Association for Single Wall Carbon Nanotubes)	2011	Daphnia magna Reproduction Test		Mitsubishi Chemical Medience Corp.	A110431			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 211 (Daphnia magna Reproduction 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:	Super Growth SWCNT

Details on test material

Lot No. HT0209-DA000

Details on test solutions

1000mg/L stock solution was made by crushing freeze dried samle and dispersing with Tween 80
10000mg/L test water.0.0030, 0.0095, 0.030, 0.095, 0.30 mg/L solution was prepared by diluting stock
solution with test water. Concentrations were not analysed. Elendt M4 medium recommended by
OECD TG 211 was used as dilution water.

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

Source: National Institute for Environmental Studies

Study design

Test type

semi-static

Water media type

freshwater

Total exposure duration

21 d

Test conditions

Hardness

<250 mg CaCO₃ / L

Test temperature

20±1°C

pH

6~9 (variation<1.5)

Dissolved oxygen

>3mg/L no aeration

Nominal and measured concentrations

0.01, 0.032, 0.10, 0.32, 1.0 mg/L (nominal)

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

Reference substance (positive control)

no

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	ca. 0.32 mg/L	nominal	test mat.		
21 d	LOEC	ca. 1 mg/L	nominal	test mat.		
21 d	EC50	> 1 mg/L	nominal	test mat.		
21 d	LC50	ca. 0.62 mg/L	nominal	test mat.		

Any other information on results incl. tables

Table8. 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	17.9	17.9	17.9	17.9	56.6	56.6	87.0	87.0	87.0	87.0	102.3	102.3	102.3	130.4
Solvent control	--	7.6	15.7	15.7	44.2	44.2	54.2	67.5	100.0	100.0	101.3	101.3	103.9	103.3	108.0	106.9
1	0.010	5.4	14.9	14.9	35.3	35.3	58.0	71.4	106.9	106.9	104.7	104.7	103.8	103.8	104.9	106.9
2	0.032	9.8	14.9	14.9	44.3	44.3	55.0	84.1	104.1	104.1	102.8	102.8	103.9	103.4	105.7	108.0

3	0.1 0	1 1 0	1 6 8	1 6 8	4 9 3	4 9 3	5 6 6	9 1 2	1 0 3	1 0 3	1 3 1	1 3 1	1 3 8	1 4 3	1 7 2	1 7 2
4	0.3 2	8 3	1 1 7	1 1 7	4 6 0	4 6 0	4 8 7	8 6 1	9 0 9	9 0 9	1 2 5	1 2 5	1 2 8	1 3 8	1 5 9	1 6 1
5	1.0	0 0	1 3 5	1 3 5	1 3 5	1 3 5	3 5 5	3 5 5	5 4 0	5 4 0	5 4 0	5 4 0	8 2 0	8 2 0	1 0 2	1 0 2

Table9. Reproduction Rate, Inhibition Rate and Results of Statistical Comparison

Test Group	Nominal Conc. (mg/L)	Cumulative Number of Offsprings										Mean	S.D.	Reprod. Rate (%)	Inhib. Rate	Signif. diff.
		Vessel No.														
		1	2	3	4	5	6	7	8	9	10					
Control	--	133	137	135	D	79	138	130	138	141	143	130.4	19.7	--	--	--
Solvent control	--	125	146	145	149	180	163	171	184	179	167	160.9	19.1	--	--	--
1	0.010	154	139	166	193	150	D	174	149	161	162	160.9	15.9	100.0	0.0	-
2	0.032	137	156	165	183	189	138	187	171	D	186	168.0	20.5	104.4	-4.4	-
3	0.10	179	173	183	159	150	187	141	186	188	174	172.0	16.6	106.9	-6.9	-

4	0.3 2	1 0 6	D	1 6 7	1 6 1	1 7 7	1 6 7	1 6 9	1 7 3	1 6 4	1 6 7	16 1. 2	2 1. 2	100 .2	- 0.2	-
5	1.0	1 0 2	D	D	D	D	D	D	D	1 0 2	D	10 2. 0	--	63. 4	36. 6	+

Equation $B = A/A \text{ solvent control} > 100$

$C = 100 - B$

D: Not included for calculation because the parent 1 Daphnia was dead during a 21-day exposure period.

-: Indicates no significant difference from the solvent control

*: Indicates a significant difference ($\alpha=0.05$) from the solvent control

(There was no sign in this test)

**: Indicates a significant difference ($\alpha=0.01$) from the Solvent control

(There was no sign in this test.)

+: Statistical analysis were performed except Conc.5 which was 80% of cumulative mortality of parent Daphnia. S significant

There is no difference ($d=0.05$) between the concentration groups except Conc. 5 and the solvent control

6.1.5 Toxicity to aquatic algae and cyanobacteria

Endpoint study record: Nikkiso SWCNT Toxicity to aquatic algae and cyanobacteria.001

Administrative Data

other:OECD/WPMN

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

The test was conducted under GLP 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	Algae Growth Inhibition Test of Nikkiso SWCNT using Pseudokirchneriella subcapitata		CERI	95401			

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:	Nikkiso SWCNT

Vehicle

yes

Test organisms**Test organisms (species)**

Pseudokirchnerella subcapitata

Details on test organisms

Obtained from American Type Culture Collection (1995) Strain No. ATCC 22662

Study design

Test type

static

Water media type

freshwater

Total exposure duration

72 h

Test conditions

Test temperature

23.2-23.4 °C

pH

7.9-8.0

Nominal and measured concentrations

Test substance; 10, 3.2, 1.0, 0.32, 0.10 mg/L

Dispersing agent; 100 mg/L

Details on test conditions

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

Light intensity; 90-94 $\mu\text{E}/\text{m}^2/\text{s}$

Continuously rotated shaking culture

Control; no test substance and dispersing agent,

Vehicle control; dispersing agent only

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

Any other information on materials and methods incl. tables

Value of biomass at each time

- a. The value based on the measured value of pre-culture
- b. The minimum cell growth in control (biomass at the end of exposure / biomass at the start of exposure) $270 / 1.8 = 150$
- c. The minimum cell growth in vehicle control (biomass at the end of exposure / biomass at the start of exposure) $310 / 1.8 = 172$

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				
72 h	NOEC	ca. 0.32 mg/L				

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 ^a	24 hours	48 hours	72 hours
Control	A	1.8	12	75	280
	B	1.8	12	72	280
	C	1.8	13	68	270 ^b
	Mean	1.8	12	72	280
	SD	0	0.48	3.2	8.0
Vehicle control	A	1.8	14	81	310 ^c
	B	1.8	13	83	320
	C	1.8	15	87	320
	D	1.8	14	83	340
	E	1.8	14	85	330
	F	1.8	13	83	330

	Mean	1.8	14	83	330
	SD	0	0.82	2.2	12
0.10	A	1.8	14	80	310
	B	1.8	13	77	310
	C	1.8	14	79	320
	Mean	1.8	14	79	310
	SD	0	0.36	1.6	9.2
0.32	A	1.8	14	80	310
	B	1.8	13	83	330
	C	1.8	14	82	320
	Mean	1.8	14	82	320
	SD	0	0.33	1.6	7.4
1.0	A	1.8	12	70	280
	B	1.8	12	69	250
	C	1.8	13	73	270
	Mean	1.8	12	71	270
	SD	0	0.41	2.0	17
3.2	A	1.8	8.3	45	170
	B	1.8	7.9	40	170
	C	1.8	7.5	39	170
	Mean	1.8	7.9	41	170
	SD	0	0.39	3.6	3.9
10	A	1.8	4.1	12	33
	B	1.8	4.1	12	34
	C	1.8	3.9	10	32

	Mean	1.8	4.0	12	33
	SD	0	0.098	1.1	1.1

a The value based on the measured value of pre-culture

b The minimum ce11 growth in control (biomass at the end of exposure / biomass at the start of exposure)
 $270 / 1.8 = 150$

c The minimum ce11 growth in vehicle control (biomass at the end of exposure / biomass at the start of exposure)
 $310 / 1.8 = 172$

Table4. Growth rate and growth inhibition

Nominal Concentration (mg/L)	No.	Growth rate (0-3d)	Inhibition rate (%)
Control	A	1.68	2.9
	B	1.68	2.8
	C	1.66	3.8
	Mean	1.67	3.2
	SD	0.00980	0.57
Vehicle control	A	1.71	--
	B	1.73	--
	C	1.72	--
	D	1.74	--
	E	1.74	--
	F	1.74	--
	Mean	1.73	--
	SD	0.0120	--
0.10	A	1.71	1.0
	B	1.71	1.1
	C	1.73	0.10
	Mean	1.72	0.74

	SD	0.00971	0.56
0.32	A	1.72	0.81
	B	1.73	0.018
	C	1.73	0.045
	Mean	1.72	0.29
	SD	0.00777	0.45
1.0	A	1.68	3.0
	B	1.64	5.3
	C	1.67	3.3
	Mean	1.66	3.9
	SD	0.0218	1.3
3.2	A	1.51	12
	B	1.51	13
	C	1.52	12
	Mean	1.51	12
	SD	0.00767	0.44
10	A	0.966	44
	B	0.980	43
	C	0.957	45
	Mean	0.968	44
	SD	0.0115	0.66

Overall remarks, attachments

Illustration (picture/graph)

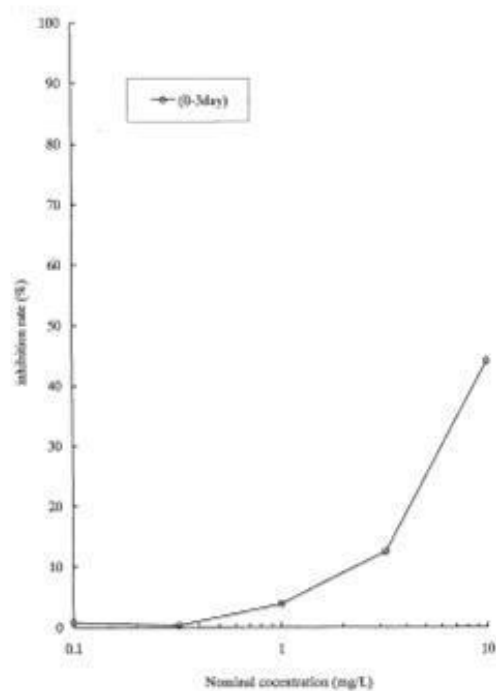


Figure 1 Concentration-response curve.

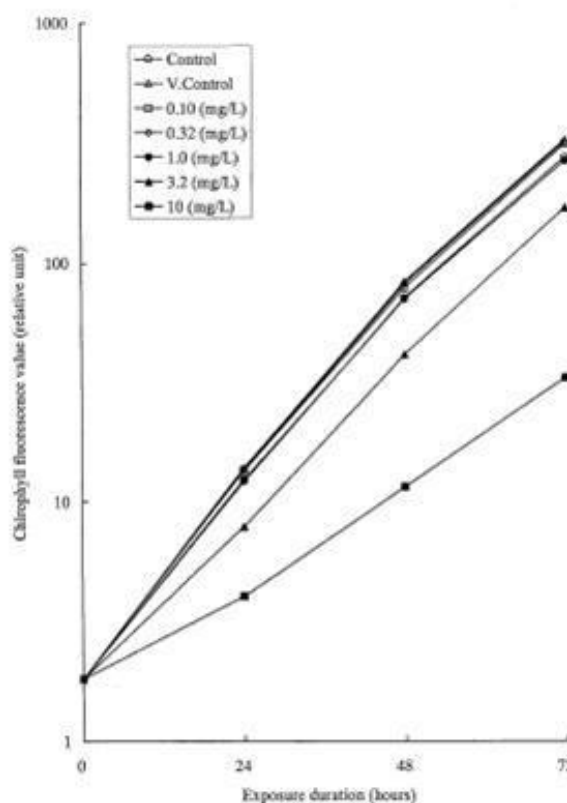


Figure 2 Growth curve.

Endpoint study record: Super Growth SWCNT Toxicity to aquatic algae and cyanobacteria.001

Administrative Data

other:OECD/WPMN

Purpose flag

key study

Study result type

experimental result

Reliability

1 (reliable without restriction)

Rationale for reliability incl. deficiencies

The test was conducted under GLP 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	Algae Growth Inhibition Test of Super Growth SWCNT using Pseudokirchneriella subcapitata		CERI	95405			

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:	Super Growth SWCNT

Vehicle

yes

Test organisms**Test organisms (species)**

Pseudokirchnerella subcapitata

Details on test organisms

Obtained from American Type Culture Collection (1995) Strain No. ATCC 22662

Study design**Test type**

static

Water media type

freshwater

Total exposure duration

72 h

Test conditions***Test temperature***

22.2-22.3 °C

pH

7.8-8.0

Nominal and measured concentrations

Test substance; 10, 3.2, 1.0, 0.32, 0.10 mg/L

Dispersing agent; 100 mg/L

Details on test conditionsOECD culture, Initial biomass concentration: 0.75×10^4 cells/mL,Light intensity; 86-88 $\mu\text{E}/\text{m}^2/\text{s}$

Continuously rotated shaking culture

Control; no test substance and dispersing agent,

Vehicle control; dispersing agent only

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

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				
72 h	NOEC	ca. 0.32 mg/L				

Any other information on results incl. tables

Value of biomass at each time

Nominal Concentration (mg/L)	No.	Chlorophyll fluorescence value (elative unit)			
		0 hour ^a	24 hours	48 hours	72 hours
Control	A	1.8	9.2	44	160 ^b
	B	1.8	9.5	51	200
	C	1.8	11	47	180
	Mean	1.8	9.8	48	180
	SD	0	0.73	3.6	17
Vehicle control	A	1.8	10	61	240
	B	1.8	11	59	230
	C	1.8	11	55	200 ^c
	D	1.8	11	62	220
	E	1.8	11	59	220
	F	1.8	11	55	200 ^c
	Mean	1.8	11	58	220
	SD	0	0.38	3.0	15
0.10	A	1.8	11	61	220
	B	1.8	12	64	220
	C	1.8	11	59	210
	Mean	1.8	12	61	220
	SD	0	0.77	2.9	3.2
0.32	A	1.8	11	59	210
	B	1.8	11	62	220
	C	1.8	12	54	200
	Mean	1.8	11	58	210
	SD	0	0.50	3.8	10

1.0	A	1.8	8.2	50	160
	B	1.8	8.9	52	180
	C	1.8	8.6	48	170
	Mean	1.8	8.6	50	170
	SD	0	0.36	2.3	5.7
3.2	A	1.8	6.1	33	110
	B	1.8	6.2	33	100
	C	1.8	6.1	31	96
	Mean	1.8	6.2	32	100
	SD	0	0.071	1.1	5.1
10	A	1.8	2.4	8.8	24
	B	1.8	2.4	9.4	25
	C	1.8	2.6	8.7	23
	Mean	1.8	2.5	9.0	24
	SD	0	0.082	0.36	0.72

a: The value based on the measured value of pre-culture

b: The minimum ce11 growth in contr01(biomass at the end of exposure / biomass at the start of exposure) $160 / 1.8 = 89$

c: The minimum ce11 growth in vehicle contr01 (biomass at the end of exposure /biomass at the start of exposure) $200 / 1.8 = 111$

Growth rate and growth inhibition

Nominal Concentration (mg/L)	No.	Growth rate (0-3d)	Inhibition rate (%)
Control	A	1.50	5.7
	B	1.56	1.8
	C	1.52	4.4

	Mean	1.53	3.9
	SD	0.0315	2.0
Vehicle control	A	1.62	-
	B	1.61	-
	C	1.57	-
	D	1.60	-
	E	1.60	-
	F	1.56	-
	Mean	1.59	-
	SD	0.0235	-
0.10	A	1.60	-0.43
	B	1.59	-0.059
	C	1.59	0.18
	Mean	1.59	-0.10
	SD	0.00483	0.30
0.32	A	1.58	0.69
	B	1.59	0.10
	C	1.56	2.1
	Mean	1.58	0.97
	SD	0.0164	1.0
1.0	A	1.50	5.7
	B	1.52	4.4
	C	1.51	5.3
	Mean	1.51	5.1
	SD	0.0112	0.71

3.2	A	1.35	15
	B	1.35	15
	C	1.32	17
	Mean	1.34	16
	SD	0.0169	1.1
10	A	0.856	46
	B	0.869	45
	C	0.850	47
	Mean	0.858	46
	SD	0.00994	0.62

Overall remarks, attachments

Illustration (picture/graph)

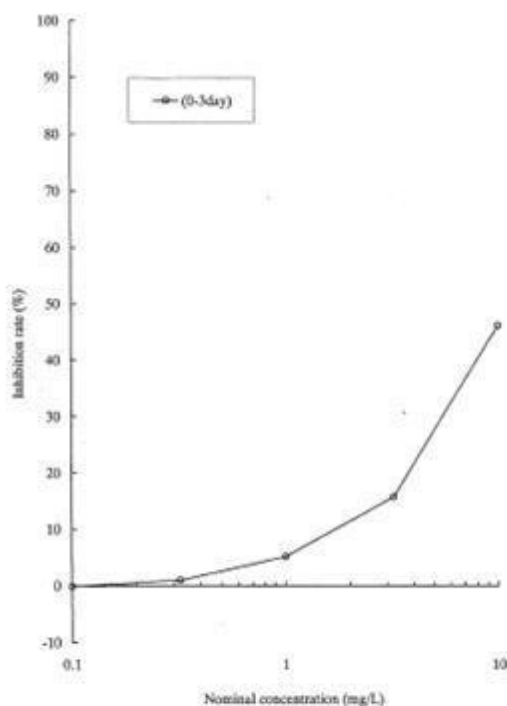


Figure 1 Concentration-response curve.

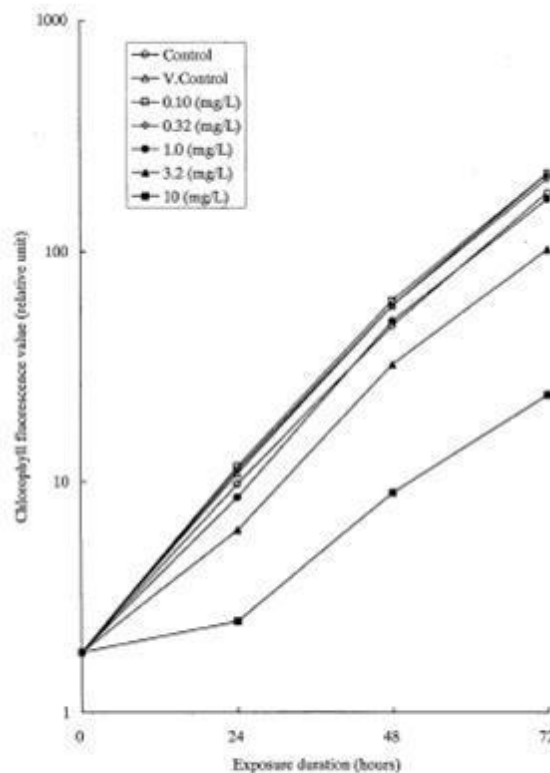


Figure 2 Growth curve.

Endpoint study record: Sigma-Aldrich SWCNT Toxicity to aquatic algae and cyanobacteria.001

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Meets generally accepted scientific standards, well documented and acceptable for assessment

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Blaise et al.	2008	Ecotoxicity of selected Nano-Materials to Aquatic Organisms	Env. Toxicol., 23: 591-598					

Materials and methods**Test materials****Test material identity**

Identifier	Identity
other: Sigma-Aldrich	

Details on test material

SWCNTs were obtained from Sigma-Aldrich (no. 519308) 50-70% SWCNTs are in bundles with diameter of 1.2. – 1.5 nm and length of 2-5 µm.

Details on test solutions

A stock solution of 500 ml/L of SWCNTs (nominal concentration) was prepared in purified water by rotator mixing at room temperature (24 hours at 12 rpm), and then filtered through a 0.22 µm cellulose membrane. Actual concentrations tested on algae were not reported.

Test organisms**Test organisms (species)**

Pseudokirchnerella subcapitata

Details on test organisms

Test organism: algae, microorganisms, fish hepatocytes, & invertebrates

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

Growth inhibition in *Pseudokirchneriella subcapitata* at a reported nominal concentration of 1.04 mg/L, and found no effects for other organisms.

Endpoint study record: Rice HPR 145.1 SWCNT Toxicity to aquatic algae and cyanobacteria.001**Administrative Data**

Purpose flag supporting study

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Meets generally accepted scientific standards, well documented and acceptable for assessment

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Toun et al.	2011	Mitigation of the impact of single-walled carbon nanotubes on a fresh green algae: <i>Pseudokirchneriella subcapitata</i>	Nanotoxicology, 2012, 6(2),161-72 (Epub 2011 Mar 21)					

Materials and methods**Principles of method if other than guideline**

The algal growth procedure used in this study was adapted from USEPA's *P. subcapitata* 96-h growth inhibition method 1003.0 (USEPA 2002).

GLP compliance

no data

Test materials

Test material identity

Identifier	Identity
other: Rice HPR 145.1	

Test material form

nanomaterial

Details on test solutions

First, 40 mg of SWNTs are suspended in 200 mL of a 1% (m/v) gum Arabic solution. The mixture was homogenized using a high-shear IKA T-25 Ultra-Turrax mixer, followed by ultrasonication with a Misonix S3000 for 10 min. These steps were followed by centrifugation at 20,000 rpm for 2.5 hrs. SWNTs were remaining suspended in the supernatant.

Test organisms

Test organisms (species)

Pseudokirchnerella subcapitata

Details on test organisms

The green algae *Selenastrum capricornutum*, renamed *Pseudokirchneriella subcapitata*, was used in all bioassays (US-EPA, 2002). The sensitivity of this algal species to toxicants may equal or even surpass that of the 48-hr *Ceriodaphnia dubia* acute test.

Study design

Test type

static

Water media type

freshwater

Test conditions

Details on test conditions

Pure cultures of *P. subcapitata* were cultivated under constant light exposure at room temperature. In the first series of experiments, the USEPA *P. subcapitata* 96-hr growth inhibition method 1003.0 was used

(USEPA 2002). The second set of experiments used long-term exposure studies (up to 12 days), with SWNTs introduced into culture media at different points along the growth curve. Overall, dose-exposure studies were conducted under sterile conditions in 150 mL Erlenmeyer flasks, and the algal growth assessed in both control (no SWNT added) and treatment (SWNTs added) containers over time.

Any other information on materials and methods incl. tables

An example of collected AFM images and the determined length distribution of the nanotubes is shown in Figure 1. GA-suspended SWNTs exhibited a length distribution ranging from 300–1200 nm. Levels of metal impurities in SWNT-suspensions determined quantitatively by thermo-gravimetric analysis and qualitatively by ICP-AES averaged 13.4% and 17 ppm, respectively.

Prior to investigating the biological effects of SWNTs, the toxicity of common surfactants (e.g. gum Arabic, SDBS, SDS, sodium-cholate, Triton X-15, Triton X-100) to *P. subcapitata* was first determined in culture media without SWNT addition. From these experiments, only gum Arabic and sodium cholate were found to be non-toxic to *P. subcapitata*. Further studies were then conducted using SWNTs suspended in gum Arabic. For the latter, dose-exposure studies were designed to study the effect of:

- i. increasing SWNT concentrations on *P. subcapitata*;
- ii. increasing gum Arabic concentrations (while SWNT concentration is maintained constant) on *P. subcapitata*; and
- iii. addition of toxic SWNT level to algal culture at different points of the growth curve.

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	NOEC	> 0.5 mg/L				

Overall remarks, attachments

Remarks on results including tables and figures

In these studies, changes in algal biomass and cell morphology associated with specific SWNT-treatments were monitored, and the mechanisms of the different biological responses investigated through a combination of biochemical and spectroscopic methods. Initially, a significant algal growth inhibition was observed after 96 hours in culture media containing 0.5 mg SWNT/L and with a final GA concentration of 0.023% (v/v). (Figure 2) Measured trends of glutathione concentrations in culture media and the presence of high levels iron-based impurities in used SWNTs pointed to the generation of ROS and oxidative stress as main cause of growth inhibition. TEM observations of control and SWNT-treated algal cells showed clear morphological impacts on the latter. However, this toxic effect was completely eliminated when GA concentrations were brought to levels 0.046%, or when the cells were allowed to grow for up to 2-weeks in the presence of the above toxic combination of SWNT and GA concentrations.

Obtained results point to the possibility of mitigating the toxicity of SWNTs through manipulation of suspension preparation methods, while maintaining the physicochemical properties sought from these nanotubes.

Illustration (picture/graph)

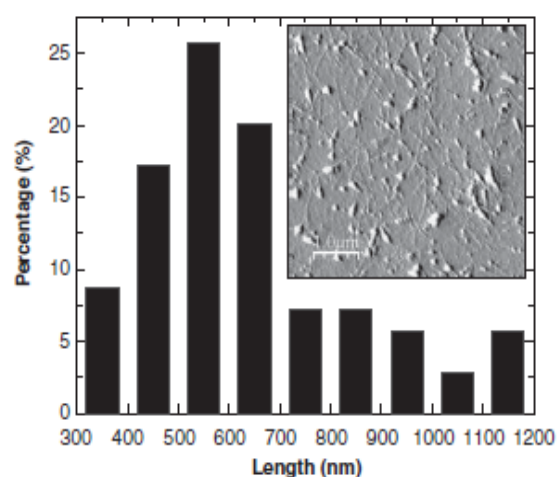


Figure 1. Length distributions of individual single walled carbon nanotubes (SWNTs) suspended in 1% gum Arabic (GA) determined by analysis of AFM image (see inset).

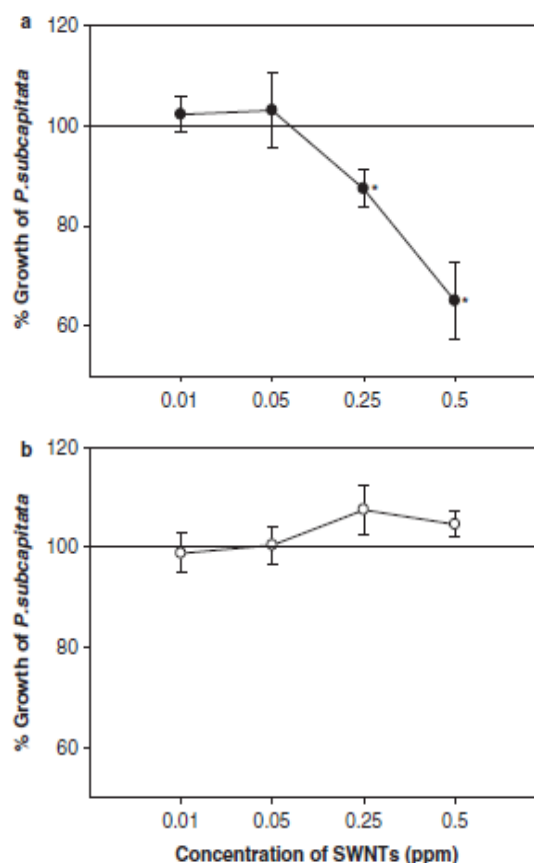


Figure 2. Effect of increasing concentrations of SWNTs on the growth of *P. subcapitata* in a standard 96-h chronic algal assay. The final concentration of gum Arabic (GA) used as surfactant to suspend SWNTs was adjusted in all culture media to a final concentration of 0.023% (1a), and 0.046% (1b). The horizontal line represents the growth of *P. subcapitata* in control culture media (i.e., culture media containing only GA but no SWNTs). Growth rates obtained from controls were assigned a value of 100% and biological responses measured in treated samples normalized to the control. (*) indicates a significant difference as compared to controls ($\alpha < 0.05$).

6.2 Sediment toxicity

Endpoint study record: SWeNT SWCNTs and Nikkiso SWCNT;Sediment toxicity.001

Administrative Data

other:OECD/WPMN

Purpose flag supporting study

Study result type other: being conducted **Study period** -2013

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Diamond D. Ho K.	2013			US EPA				

Materials and methods

Test materials

Test material identity

Identifier	Identity
other:	SWeNT SG65 (SWCNT)
other:	SWeNT SG76 (SWCNT)
other:	SWeNT CG100 (SWCNT)
other:	Nikkiso SWCNT

Details on test material

SWeNT SG65 (SWCNT):Weight: 85-99% SWCNT,
Diameter Given from Manufacturer: 0.8 +/- 0.1nm,
Experimentally Derived via Fluorescence: Mean diameter (0.793nm),
Impurities: Molybdenum and Cobalt ◇
Weight: 1-15%,

Single wall carbon nanotubes, with various amounts of molybdenum and cobalt, black powder, solid, insoluble to water, pH 4-10SWeNT SG76 (SWCNT):

Weight: 85-99% SWCNT,

Diameter Given from Manufacturer: 0.93 +/- 0.27nm,

Experimentally Derived via Fluorescence: Mean diameter (0.893nm),

Impurities: Molybdenum and Cobalt ◇

Weight: 1-15%,

Single wall carbon nanotubes, with various amounts of molybdenum and cobalt, black powder, solid, insoluble to water, pH 4-10SWeNT CG100 (SWCNT):

Weight: 85-99% SWCNT,

Diameter Given from Manufacturer: 0.87 +/- 0.3nm,

Experimentally Derived via Fluorescence: Meandiameter (0.828nm),

Impurities: Molybdenum and Cobalt ◇

Weight: 1-15%,

Single wall carbon nanotubes, with various amounts of molybdenum and cobalt, black powder, solid, insoluble to water, pH 4-10 Nikkiso SWCNT: Principal material

Test organisms

Test organisms (species)

Ampelisca abdita

Details on test organisms

Americamysis bahia is also used.

Test conditions

Any other information on materials and methods incl. tables

Materials were spiked into marine sediments 0.1, 1.0 and 10ppm using 2% sodium deocycholate SDC.

Acute assays performed using marine invertebrates *Ampelisca abdita* (amphipod) and *Americamysis bahia* (mysid shrimp).

Overall remarks, attachments

Remarks on results including tables and figures

Data analysis underway. Publication date 2013.

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

Endpoint study record: Carbon Solution SWCNT Toxicity to terrestrial plants.001

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Meets generally accepted scientific standards, well documented and acceptable for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Canas et al.	2008	Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species	Environ Toxicol Chem. 2008 Sep;27(9):1922-31					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: recommended by the US EPA for phytotoxicity studies	

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: Carbon Solutions, Inc	

Details on test material

P3-SWCNTs were purchased from Carbon Solutions, Inc. 80-90% purity, 3% metal content by weight.

Test organisms

Test organisms

Details on test organisms Tomato, Onion, Cucumber

Test conditions

Details on test conditions

CNT suspensions were prepared by mixing 100 mg of CNTs with 4 ml of water and then sonicating for 30 min. Then, an additional 16 ml of water was added, and sonication continued for an additional 90 min. to provide the stock solution. Dilutions of this stock solution with water were made to produce test concentrations of 56, 315, and 1,750 mg/L of CNTs (nominal concentrations). A water-only control was also run, and each plant (which was grown from seed in the absence of CNTs until most generated radicals) was exposed to 1 ml of test solution in a petri dish with filter paper), and examined at 24 and 48 hours. Regressions were run comparing root elongation to CNT concentrations over the two time courses.

Results and discussions

Details on results

Significant decreases in root elongation were seen in tomato plants at both 24 and 48 hrs; significant increases in root elongation were seen in onions at 24 and 48 hrs, and a significant increase in root elongation was seen in cucumbers at 24 hrs. Electron microscopy showed extensive CNT sheets covering root surfaces which could affect necessary plant and root functions that would not be evident in root elongation tests of this duration.

6.3.4 Toxicity to soil microorganisms

Endpoint study record: Nikkiso SWCNT Toxicity to soil microorganisms.001

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted under GLP 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	Nitrogen Transformation test for Soil Microorganisms of Nikkiso SWCNT		Mitsubishi Chemical Medience Corp.	A100700			

Materials and methods**Test guideline**

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

Test organisms**Test organisms (inoculum)**

soil

Study design**Total exposure duration**

28 d

Test conditions***Test temperature***

20+/-2°C

Moisture content

50+/-5% for maximum content

Organic carbon content (% dry weight)

0.9%

Nitrogen content (% dry weight)

0.122%

Any other information on materials and methods incl. tables

total soil organic carbon)

Dose/concentration level: Test substance 1000 mg/kg dry-soil; Substrate 5 g/kg dry-soil; Reference substance 0.1,

0.32, 1, 3.2, 10 mg/kg dry-soil

Test Item: Rate of nitrate formation

Other information: Substrate young alfalfa powder;

Reference substance 2-Chloro-5-(trichloromethyl) pyridine

Exposure duration: 28 days, Dark condition,

Sampling: 0, 7, 14, 21, 28 days after initiation of the test

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				

Any other information on results incl. tables

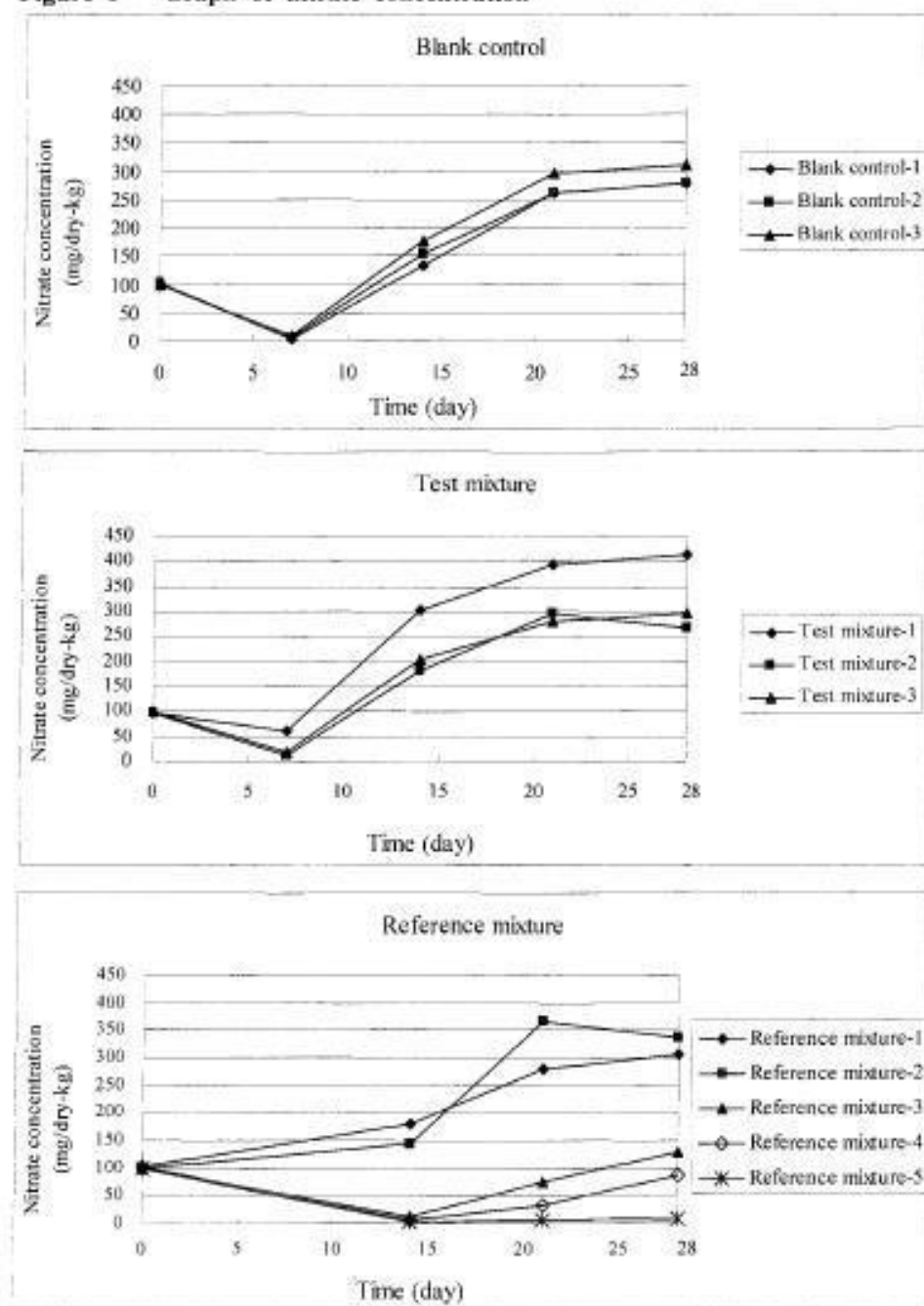
Nitrate formation rate

		Nitrate formation rate (mg/dry-kg/day)		
		B	C	D
		7-14 day	14-21 day	21-28 day
Blank control	1	18.5	18.5	2.3
	2	20.8	15.6	2.2
	3	24.2	17.0	2.2
	Average	21.2	17.0	2.2
Test mixture	1	34.2	13.2	3.1
	2	24.1	16.4	-4.3
	3	26.3	10.7	2.6
	Average	28.2	13.4	0.4
Reference mixture	1	-	14.2	3.9
	2	-	31.5	-4.4
	3	-	9.1	7.6
	4	-	4.0	7.5
	5	-	0.4	0.7

Overall remarks, attachments

Illustration (picture/graph)

Figure 1 Graph of nitrate concentration



Endpoint study record: Super Growth SWCNT Toxicity to soil microorganisms.001**Administrative Data**

other:OECD/WPMN

Purpose flag key study**Study result type** experimental result**Reliability** 1 (reliable without restriction)**Rationale for reliability incl. deficiencies** The test was conducted under GLP 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	Nitrogen Transformation test for Soil Microorganisms of Super Growth SWCNT		Mitsubishi Chemical Medicine Corp.	A100432			

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	OECD Guideline 216 (Soil Microorganisms: Nitrogen Transformation 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:	Super Growth SWCNT

Test organisms

Test organisms (inoculum)

soil

Study design

Total exposure duration

28 d

Test conditions

Test temperature

20+/-2°C

Moisture content

50+/-5% for maximum content

Organic carbon content (% dry weight)

0.9%

Nitrogen content (% dry weight)

0.122%

Any other information on materials and methods incl. tables

Species: Soil texture clay loam, sand content 53.5 %, pH 5.4, OC content 0.9%, Biomass carbon 88

mg/kg (1 %of total soil organic carbon)

Dose/concentration level: Test substance 1000 mg/kg dry-soil; Substrate 5 g/kg dry-soil;

Reference substance 0.1, 0.32, 1, 3.2, 10 mg/kg dry-soil

Test Item: Rate of nitrate formation

Other information: Substrate young alfalfa powder;

Reference substance 2-Chloro-5-(trichloromethyl) pyridine

Exposure duration: 28 days, Dark condition,

Sampling: 0, 7, 14, 21, 28 days after initiation of the test

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				

Any other information on results incl. tables

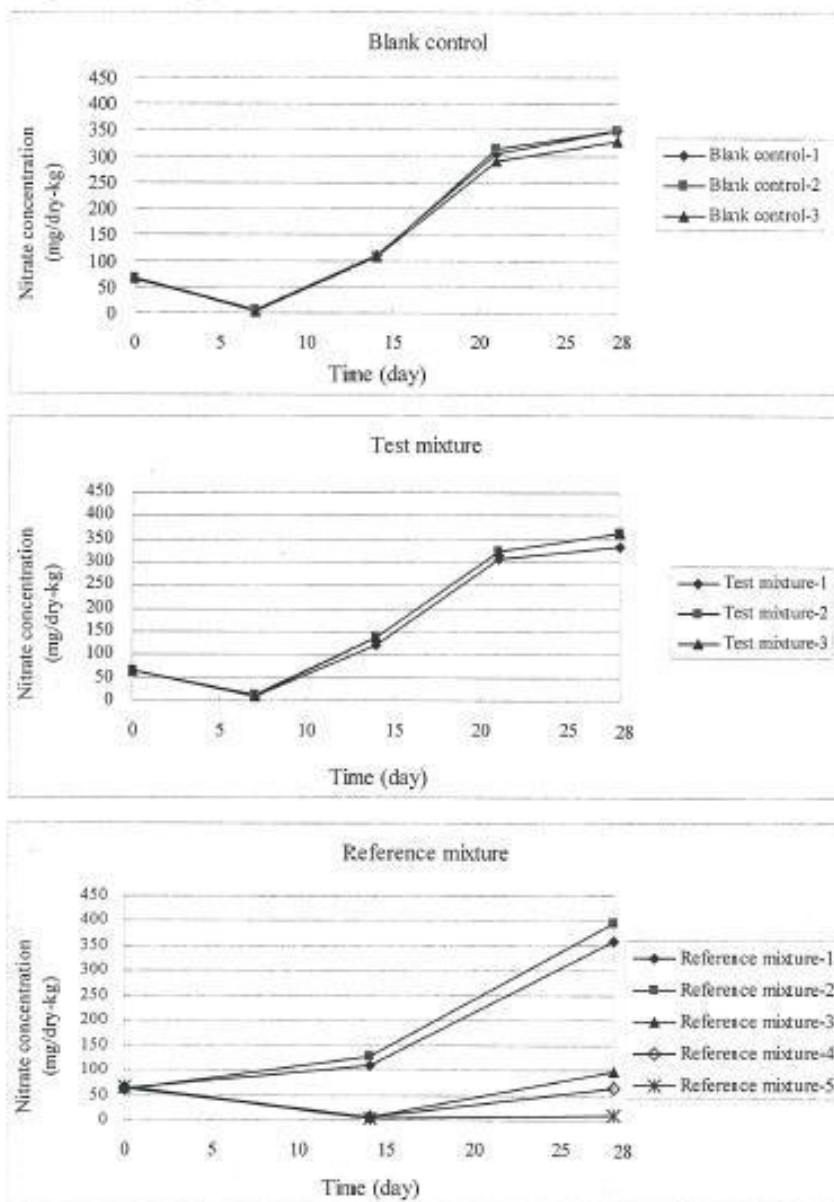
Nitrate formation rate

		Nitrate formation rate (mg/dry-kg/day)		
		B	C	D
		7-14 day	14-21 day	21-28 day
Blank control	1	15.0	27.6	6.3
	2	14.4	29.3	5.2
	3	14.7	26.3	5.5
	Average	14.7	27.7	5.7
Test mixture	1	15.6	26.8	3.7
	2	17.8	26.4	5.6
	3	18.3	26.3	5.5
	Average	17.2	26.5	4.9

Overall remarks, attachments

Illustration (picture/graph)

Figure 1 Graph of nitrate concentration



6.4 Biological effects monitoring**6.5 Biotransformation and kinetics****6.6 Additional ecotoxicological information**

Endpoint study record: Nikkiso SWCNT Respiration Inhibition test; Additional ecotoxicological information.001

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted under GLP 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	Respiration Inhibition test of Nikkiso SWCNT using activated sludge		CERI	95400			

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	other guideline: OECD TG 209	

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 SWCNT

Any other information on materials and methods incl. tables

Dose/concentration level: Test substance 100 mg/L,

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

Test Item: Oxygen uptake rate calculated from dissolved oxygen concentrations

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

Temperature: 20±2°C, Exposure period: 3 hours

Results and discussions

Any other information on results incl. tables

EC50: > 100 mg/L (Test substance), 6.75 mg/L (Reference substance)

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

Oxygen consumption rate and respiratory inhibition rate

Test solution	concentration (mg/L)	O ₂ consumption. rate (mgO ₂ /L*h)	respiratory inhibition rate (%)
Test material	100	29.1	7
Control (3,5dichlorophenol)	4.00	21.1	33
	8.00	11.9	62
	16.0	9.61	69
	32.0	7.07	77
Control (blank)	-	30.7	-
	-	31.8	-

Endpoint study record: Super Growth SWCNT Respiration Inhibition test; Additional ecotoxicological information.001

Administrative Data

other:OECD/WPMN

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The test was conducted under GLP 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	Respiration Inhibition test of Super Growth SWCNT using activated sludge		CERI	95404			

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	other guideline: OECD TG 209	

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:	Super Growth SWCNT

Any other information on materials and methods incl. tables

Dose/concentration level: Test substance 100 mg/L,
Reference substance(3,5 -Dichlorophenol);4, 8, 16 and 32 mg/L
Test Item: Oxygen uptake rate calculated from dissolved oxygen concentrations
Species: Activated sludge of a sewerage treatment plant(Kurume City, Japan)
Temperature: 20±2°C,

Exposure period: 3 hours

Results and discussions

Any other information on results incl. tables

EC50: > 100 mg/L (Test substance), 6.75 mg/L (Reference substance)

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

Oxygen consumption rate and respiratory inhibition rate

Test solution	concentration (mg/L)	O ₂ consumption. rate (mgO ₂ /L*h)	respiratory inhibition rate (%)
Test material	100	31.6	0 (*)
Control (3,5dichlorophenol)	4.00	21.1	33
	8.00	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 values set as zero.

Endpoint study record: SWeNT SWCNT Bioavailability of Legacy Contaminants

Administrative Data

other:OECD/WPMN

Purpose flag supporting study

Study result type other: being conducted **Study period** -2013

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
publication	Diamond S., Ho K.	2013			US EPA				

Materials and methods

Test materials

Test material identity

Identifier	Identity
other:	SWeNT SG65 (SWCNT)

Any other information on materials and methods incl. tables

Examined the ability of SWNT 65 to mitigate the toxicity of PCDB contaminated sediment to A abdita and A bahia.

Results and discussions

Any other information on results incl. tables

SWNT 65 appear to mitigate the toxicity of a PCB contaminated sediment to A abdita and A bahia.

Overall remarks, attachments

Remarks on results including tables and figures

Data is being analyzed and papers will be published in 2013

Endpoint study record: Nano. Amor. Mat. SWCNT Aquatic organism in vitro test.001**Administrative Data****Purpose flag** supporting study**Study result type** experimental result **Study period** 2010**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
publication	Klaper et al.	2010	Functionalization impacts the effects of carbon nanotubes on the immune system of rainbow trout, <i>Oncorhynchus mykiss</i>	Aquatic Toxicol. 100: 211-217					

Materials and methods**Test materials****Test material identity**

Identifier	Identity
other: Nanostructured and Amorphous Materials	

Details on test material

Material: purchased from Nanostructured and Amorphous Materials, Inc

Any other information on materials and methods incl. tables

Test organism: trout kidney monocyte primary cell

Test method: The impact of SWCNTs on rainbow trout kidney monocyte primary cell cultures was

examined.

The SWCNTs were dispersed without solvents by stirring for 2 weeks in RNase-free water.

The gene analysis assays were conducted after 6 h or 24h exposures.

Overall remarks, attachments

Remarks on results including tables and figures

6-24 hours NOEC = 2.5 mg/L, LOEC = 5.0 mg/L

SWCNT concentrations of 5.0 µg/ml and greater resulted in IL-1B gene expression stimulation that was greater than the water control, while 2.5 µg/ml did not. The expression levels at 5.0 µg/ml in trout macrophages indicated that SWCNTs can be proinflammatory in trout if they gain access to the body.

Endpoint study record: CNI SWCNT in vitro ecotoxicological information.001

Administrative Data

Purpose flag supporting study

Study result type experimental result **Study period** 2009

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
publication	Liu et al.	2009	Carbon nanotubes as molecular transporters for walled plant cells	Nano Letters, 9: 1007-1010					

Materials and methods**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: CNI SWCNT	

Details on test material

Water-soluble SWCNTs were prepared from Hipco SWCNTs (Carbon Nanotechnologies, Inc.; length < 500 nm). SWCNTs were oxidized (concentrated sulfuric acid/nitric acid mixture; ultrasonication for 24h, filtration, and further sonication), and labeled noncovalently with fluorescein isothiocyanate (by centrifugation and sonication).

Any other information on materials and methods incl. tables

Test organism: *Nicotiana tabacum* L.cv. bright Yellow (BY-2) cells

Test Method: Cells were treated with an estimated 2, 10, 20, 40, and 80 mg/L of labeled SWCNTs for 2 hours at 26°C or 4°C.

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

Cells showed little uptake of SWCNTs at 4°C, indicating the involvement of endocytosis as a mechanism for penetration.