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THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**DOSSIER ON FULLERENES
- PART 1 -**

**Series on the Safety of Manufactured Nanomaterials
No. 48**

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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 48

**DOSSIER ON FULLERENES
- 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)*

No. 46, *Dossier on Dendrimers (2015)*

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

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

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

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

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

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

No. 54, *Dossier on Titanium dioxide (2015)*

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PREAMBLE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FOREWORD

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

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

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

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

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

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

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

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

ACKNOWLEDGMENTS

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

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1. GENERAL INFORMATION

1.1 Identification

Substance identification

Type of substance

Composition mono constituent substance

Origin element

Other names

Flags	Name Type	Name	Country	Remarks
	Trade name	Nanom Purple (Frontier Carbon Co.)		
	Trade name	C60 MER Corp.		

1.2 Composition

Substance composition

Name Degree of purity > 99.8 % (w/w)
--

1.3 Identifiers

1.4 Analytical information

1.5 Joint submission

1.6 Sponsors

1.7 Suppliers

1.8 Recipients

1.9 Product and process orientated research and development

2. CLASSIFICATION & LABELLING AND PBT ASSESSMENT

2.1 GHS

2.2 DSD - DPD

3. MANUFACTURE, USE AND EXPOSURE

3.1 Technological process

Technological process

Methods of manufacture of substance	Combustion method with sublimation purification.
--	--

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: Appearance/physical state/colour.001

Administrative Data

Study result type read-across based on grouping of substances (category approach)

Reliability 2 (reliable with restrictions)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Dresselhaus M. S. et al.,	1996	Science of Fullerenes and Carbon Nanotubes	Academy Press, 1996					
publication	AIST(Japan)	2011	Nanomaterials risk assessment Report - fullerene C60-	NEDO Project (P06041)					

Results and discussion

Physical state at 20°C and 1013 hPa

solid

Form

crystalline

Colour

black

Odour

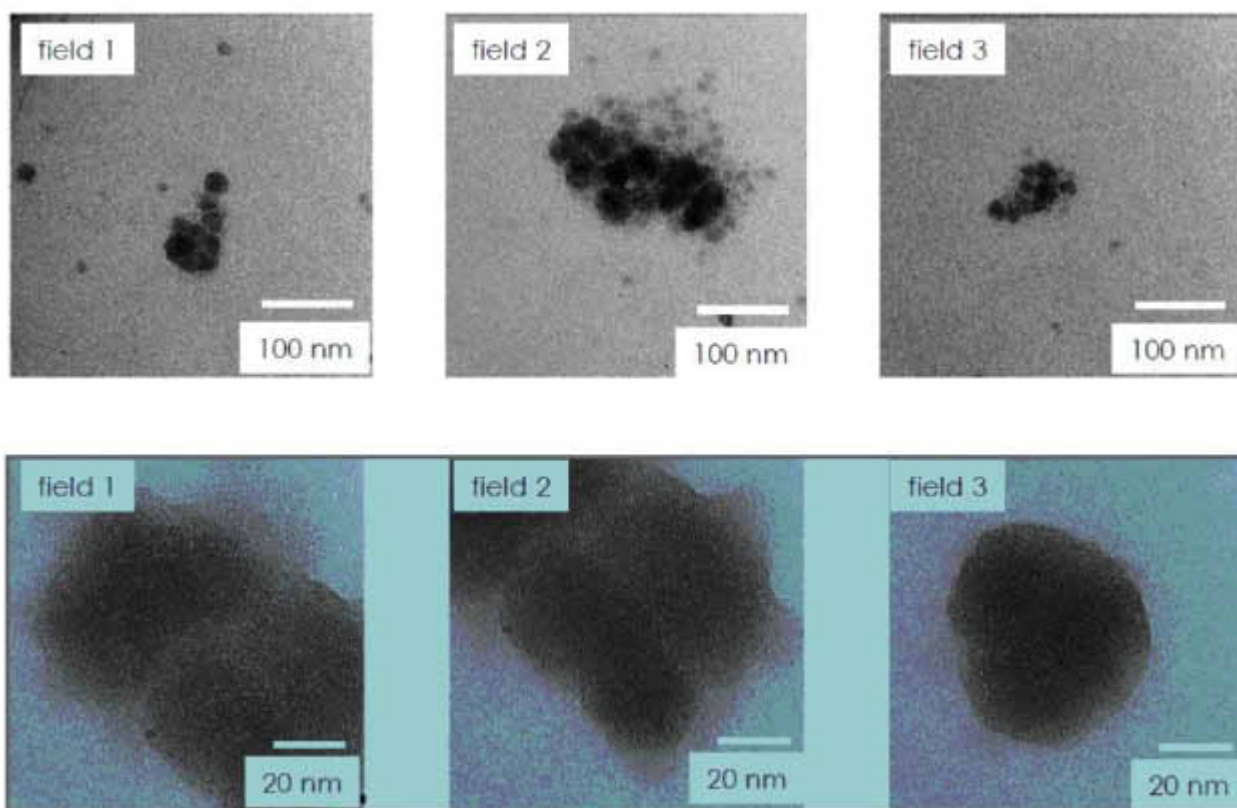
odourless

Substance type

element

Overall remarks, attachments

Illustration (picture/graph)



4.2 Melting point/freezing point***Endpoint study record: Melting point/freezing point.001*****Administrative Data****Study result type** read-across based on grouping of substances (category approach)**Reliability** 2 (reliable with restrictions)**Data source****Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	NEDO project(P06041):Research and development of a nanoparticle characterisitic evaluation technology	2010		Nanomaterials risk assessment document-Fullerene(C60)-					

Materials and methods**GLP compliance**

no data

Results and discussions**Melting / freezing point**

Melt./Freez. pt.	Atm. pressure	Decomposition	Decomp. temp.	Sublimation	Subl. temp.	Remarks
1180 °C						

4.3 Boiling point**4.4 Density****4.5 Particle size distribution (Granulometry)*****Endpoint study record: Particle size distribution (Granulometry).001*****Administrative Data****Study result type** experimental result**Reliability** 1 (reliable without restriction)**Rationale for reliability incl. deficiencies** according to TEM

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	NEDO project(P06041):Reserch and development of a nanoparticle characteristic evaluation technology	2010		Nanomaterials risk assessment document- Fullerene(C60)-	AIST(Japan)				

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

Particle size distribution of the sample used for mammalian toxicology was measured with DLS. The measured medium mass diameter ranged from 20 to 30 nm. The crystallite size consisting aggregate was obtained by the TEM observation. The medium mass diameter ranged from 20 to 30 nm.

GLP compliance

no data

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

yes

Results and discussions**Mass median diameter**

≥ 0.02 — ≤ 0.03 μm

Remarks TEM picture of fullerene crystal(picture)

4.6 Vapour pressure***Endpoint study record: Vapour pressure.001*****Administrative Data**

Study result type read-across based on grouping of substances (category approach)

Reliability 2 (reliable with restrictions)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	NEDO project(P06041)	2010		Nnomaterials risk assessment document-Fullerene(C60)-					

Results and discussions**Vapour pressure**

0.000019 mm Hg

400

0.0005 mm Hg

500

0.001 mm Hg

600 °C

4.7 Partition coefficient***Endpoint study record: Partition coefficient.001*****Administrative Data****Study result type** read-across based on grouping of substances (category approach)**Reliability** 2 (reliable with restrictions)**Data source****Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Jafvert C.T. et al.	2008	Buckminsterfullerene's (C60) octanol-water partition coefficient (Kow) and aqueous solubility.	Environ. Sci. Technol., 42 (16) p.5945-5950					

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

no

Test material identity

Identifier	Identity
	fullerene(Mer Corp.)

Results and discussions***Details on results***

The octanol-water partition coefficient:Kow is 6.67.

4.8 Water solubility***Endpoint study record: Water solubility.001*****Administrative Data**

Study result type read-across based on grouping of substances (category approach)

Reliability 2 (reliable with restrictions)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Heymann, D.			Fullerenes, Nanotubes and Nanostructurers, Vol.4 P.509-515 (1996)					

Materials and methods**GLP compliance**

no data

Test materials**Test material identity**

Identifier	Identity
	C60 was purchased from Bucky USA

Results and discussions**Water solubility**

ca. 0 mg/L

Temp. 25 °C

Overall remarks, attachments

Remarks on results including tables and figures

This endpoint was completed by read-across the existing data including above two studies.

The solubility to water of fullerenes is considered as 1.3×10^{-11} mg/l by the extrapolation of the solubility study in several alcohols.

On the other hand, the fullerenes are stably suspended in pure water by long time stirring. The surface of fullerenes is hydrated and changes from hydrophobic to hydrophilic in this condition.

Applicant's summary and conclusion

Conclusions

The solubility to water of fullerenes is considered as 1.3×10^{-11} mg/l by the extrapolation of the solubility study in several alcohols.

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

Endpoint study record: Additional physico-chemical information.001

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Kong, L., Zepp, R.	2011	Production and Consumption of Reactive Oxygen Species by Fullerenes						

Materials and methods

Endpoint investigated

other: Production and Consumption of Reactive Oxygen Species

Principles of method if other than guideline

Methods: Fullerenes are well-known photo-sensitizers, with the ability to produce singlet oxygen (1O_2) on irradiation. The formation of ROS by fullerenes typically follows two pathways: electron transfer (type I) resulting in the production of O_2^- and energy transfer (type II) leading to the generation of 1O_2 . Efficient photo-induced singlet oxygen production was observed on irradiation of molecular C60 in air-saturated organic solvents as a result of energy transfer from C60 triplet states to molecular oxygen (Arbogast et al 1991). Fullerenes form aggregates in water, and these aggregates have photochemical properties different from those of molecular fullerenes. For example, although molecular C60 photo-produces singlet oxygen efficiently, no ROS production was detected from C60 water clusters (aqu/nC60) by measuring the concentration of 1O_2 and O_2^- using electron paramagnetic resonance (EPR) (Lee et al 2008). This result was attributed to the enhanced decay of excited triplet-state C60 (precursors of ROS) by the formation of aggregates in water. Another study also showed no photosensitized generation of 1O_2 and O_2^- in bacterial media and no ROS-mediated damage in nC60-exposed bacteria (Lyon et al 2008). However, a later study found not only that singlet oxygen was produced in a C60 water suspension irradiated by sunlight but also that its production efficiency increased with irradiation time (Hou et al 2009). Aqueous suspensions of C60 were prepared by mixing ground C60 powder with Nanopure water on a magnetic stirring plate for at least four weeks. Stirring was stopped and the suspension was allowed to sit for 24 hours prior to subsequent filtration with 0.75 μ m glass fiber filters. A stock suspension of fullerenol was prepared according to the methods by Kong et al 2009 by adding fullerenol powder into Nanopure water and mixing for 24 hours. Both stock suspensions were adjusted to neutral pH using 0.05 M pH 7.0 phosphate buffers prior to use. An Atlas SunTest CPS solar simulator equipped with a 1-kW xenon arc lamp was used for simulated solar irradiations, and a rotating turntable reactor was used for monochromatic irradiations. The temperature was maintained at 22 $\pm$ 1 degree centigrade. A combination of Corning 0-52 and 7-37 glass filters was used to isolate the 366-nm wavelength from a Hanovia mercury vapor lamp in the MGRR, and a set of Kopp 3480 cutoff filters was used to block wavelengths shorter than 547 nm. A Shimadzu UV-2450 spectrophotometer equipped with an ISR-2200 integrating sphere attachment was used to compute

quantum yields for production of singlet oxygen and superoxide/hydroperoxyl. Transmission electron microscope (TEM) images were obtained using a Hitachi 7600 transmission electron microscope operated at 120 kV. C60 chemical analysis was done with a Dionex Ultimate 3000 HPLC system with a mobile phase acetonitrile:toluene = 20:80 at a wavelength of 333 nm. The fullereneol concentration was determined using a Perkin Elmer Lambda 35 UV/Vis spectrometer.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
	MER Corp C60

Results and discussions

Results

Results: Singlet oxygen is the lowest excited state of molecular oxygen and is one of the most active intermediates in chemical and biochemical reactions. Aqu/C60 and fullereneol suspensions were irradiated with 366 nm light in the presence of Furfuryl alcohol (FFA) which is a singlet oxygen scavenger. Furfuryl alcohol was photo-oxidized more rapidly in the pre-irradiated aqu/nC60 than in non-irradiated aqu/nC60. Fluorescence EEM was measured to detect the presence of water soluble fullerenes after then irradiation of aqu/C60. Prior to irradiation, no EEM signal was detected. After irradiation of aqu/C60, and following toluene extraction, an EEM spectrum very similar to that of irradiated fullereneol aqueous suspension was produced. Results indicate that singlet oxygen production increased as aqu/nC60 conversion to hydrophilic species increased. To understand the mechanism of singlet oxygen production in the presence of aqu/nC60 and fullereneol better, the researchers conducted FFA oxidation under simulated solar irradiation using oxygen- and air-saturated suspensions of aqu/nC60. The rate of FFA loss increased slightly with irradiation time in all these systems, whereas FFA photo-oxidation displayed first-order kinetics in the presence of fullereneol. This observation further confirms the involvement of aqu/nC60 photo-products in the photo-sensitized reactions of FFA. Fullerenes reacted slowly with singlet oxygen, but rapidly with hydroxyl radicals. These results show that environmental conditions, including light exposure and oxygen concentration, have the potential to impact the generation of toxic ROS by fullerenes. Both Buckminster fullerene and fullereneol can photosensitize the production of singlet oxygen, superoxide ions and hydrogen peroxide under environmentally relevant conditions. Aqu/nC60 generally exhibit lower photo-production efficiencies of singlet oxygen and superoxide than fullereneol, most likely as a result of the different structures of the aqu/nC60 aggregates and consequently an efficient self-quenching of C60 triplet states within the aggregates. This reduction in photo-chemical efficiency caused by the structure of C60 aggregates in water is partially offset by the production of hydroxylated, hydrophilic aqu/nC60 photo-products that sensitize the production of singlet oxygen. This latter process results in increases in $^{1}O_2$ production efficiencies with increasing light exposure. Both aqu/nC60 and fullereneol react very slowly with singlet oxygen. However, aqu/nC60 scavenges ($\bullet OH$) radicals at close to a diffusion-controlled rate and more than 12-fold more rapidly than fullereneol. Taken together, these results indicate that sensitivity to light exposure, oxygen concentration, and other environmental conditions are likely to affect greatly the

photo-sensitization of ROS and ROS scavenging properties of fullerenes and ultimately affect their roles in other physicochemical and biological processes.

4.24 Agglomeration/aggregation

Endpoint study record: Agglomeration/aggregation.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. Experiment was carried out by the reliable research insitute deficiencies (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST		Preparation and mesurement method for the sample used in nanomateial toxicity test	NEDO Project (P06041)	AIST				

Data access

data published

Materials and methods

Method

TEM

Test materials

Test material Identity

Identifier	Identity
	Nanompurple (Frontier Carbon Co.)

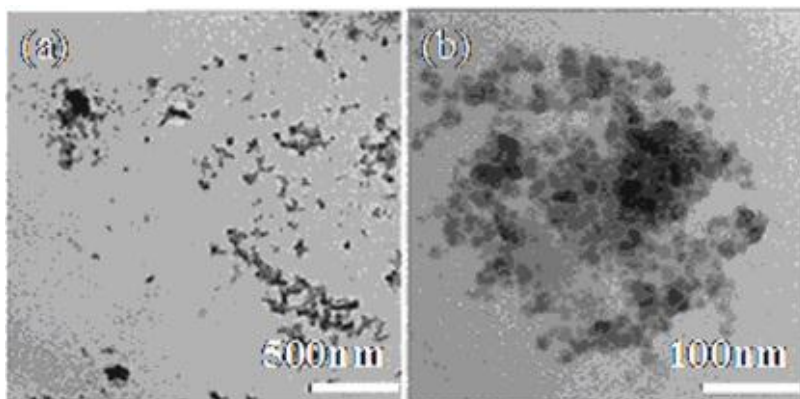
Results and discussions

Agglomerate/Aggregate diameter

Mean diameter > 500 nm

Overall remarks, attachments**Remarks on results incl. tables and figures**

The dispersed sample was observed with TEM. Primary particles of around 30 nm dia. were agglomerated in a size of about 500 nm or larger as shown in the TEM picture.

Illustration (picture/graph)**Applicant's summary and conclusion****Conclusions**

Primary particles of around 30 nm dia. were agglomerated in a size of about 500 nm.

4.25 Crystalline phase***Endpoint study record: Crystalline phase.001*****Administrative Data**

Purpose flag key study

Study result type read-across from supporting substance (structural analogue or surrogate)

Reliability 2 (reliable with restrictions)

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Fischer et al.	1993	Order and disorder in fullerene and fulleride solids	J. Phys. Chem. Solids Vol54, No. 12, pp 1725-1757					

Results and discussions

Crystalline Phase

Crystal System	cubic
Bravais lattice	face - centred cubic
Point Group	>260K
	cubic
Crystal System	
Bravais lattice	primitive cubic
Point Group	<260K

Remarks on results incl. tables

The crystalline phase of C60 is fcc (face-centered cubic) above 260K and sc (simple cubic) beneath 260K. The crystalline phase is an intrinsic property of material; therefore, this endpoint was completed by using the above established data.

4.26 Crystallite and grain size

Endpoint study record: Crystallite and grain size.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	Experiment was carried out at reliable national research center (AIST)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	AIST		Sample preparation and measuring method for toxicity test of nanomaterials	NEDO Project (P06041)	AIST				

Materials and methods

Method

transmission electron microscopy (TEM)

Test materials

Test material identity

Identifier	Identity
	nanom purple SU

Test material form

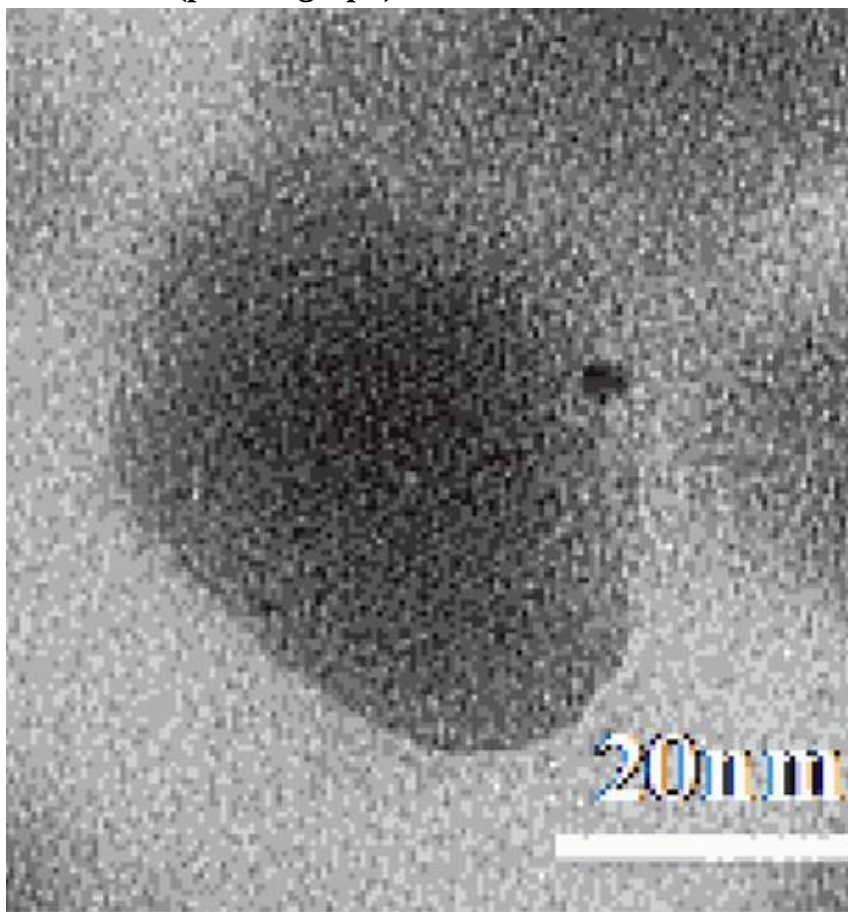
dispersion

Results and discussions

Remarks on results including tables and figures

The medium mass diameter ranged from 20 to 30 nm.

Illustration (picture/graph)



4.27 Aspect ratio/shape*Endpoint study record: Aspect ratio/shape.001***Administrative Data****4.28 Specific surface area***Endpoint study record: Specific surface area.001***Administrative Data**

Purpose flag key study

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

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies The experiment was carried out following ISO standard

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	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
	nanom purple SU

Any other information on materials and methods incl. tables

The sample was pretreated for 15 minutes at 250 Celsius degree under nitrogen gas flow. The measurement was conducted by one point BET method (adsorption gas: Nitrogen) using Macsorb HM Model-1201 (Mountech Co., Ltd.)

Results and discussions

Specific Surface Area

Specific Surface Area ca. 0.87 m²/g

4.29 Zeta potential

Endpoint study record: Zeta potential.001

Administrative Data

Purpose flag key study
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability incl. The experiment was carried out at the reliable national research center AIST
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	2009							

Materials and methods

Details on methods and data evaluation

The electrophoretic mobility of the sample used for mammalian toxicity test was measured, and zeta potential was calculated based on the results.

Test materials

Test material Identity

Identifier	Identity
	nanom purple

Results and discussions

Zeta Potential

Zeta Potential ca. -20 mV

4.30 Surface chemistry

4.31 Dustiness

Endpoint study record: Dustiness.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ogura et al.	2009	Dustiness testing of engineered nanomaterials	Isamu Ogura et al. 2009 J. Phys.: Conf. Ser. 170 012003	AIST				

Materials and methods

Methods

vortex shaker

Test materials

Test material Identity

Identifier	Identity
	Nanompurple

Any other information on materials and methods incl. tables

AIST of Japan has measured the dustiness of the principal fullerenes with Vortex Shaker Method. The fullerenes in a range of 0.5 to 1 cm³ are placed in a glass test tube. They are agitated by a laboratory vortex shaker. The number concentrations and size distributions of aerosol particles released during agitation were continuously measured using an APS (Aerodynamic Particle Sizer), an OPC (Optical Particle Counter), a SMPS (Scanning Mobility Particle Sizer) and a CPC (Condensation Particle Counter).

Results and discussions

Dustiness Index

Mean ≥ 0.65 mg/cm³

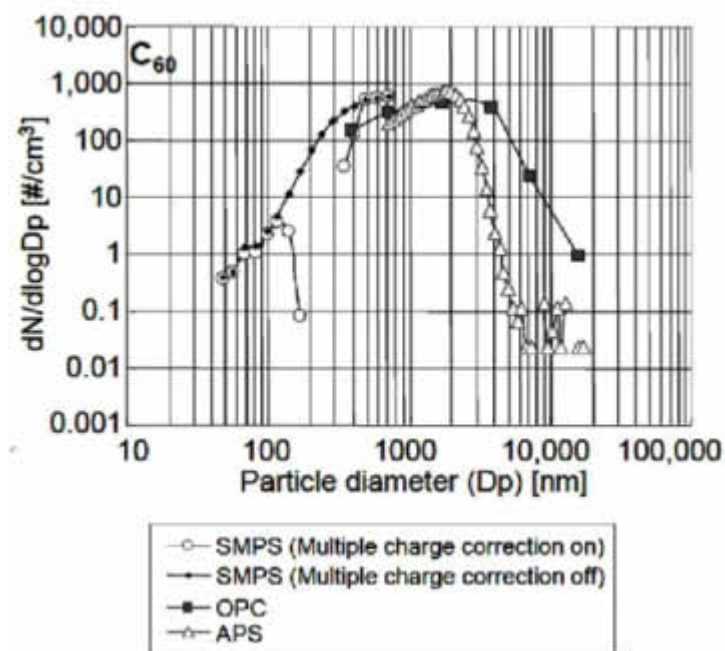
St. Deviation 1.6

Remarks on results incl. tables

Respirable particle mass concentration was 0.65 mg/m³, GSD=1.6

Overall remarks, attachments

Illustration (picture/graph)



4.32 Porosity

Endpoint study record: Porosity.001

Administrative Data

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

Data source

Reference

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

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
	nanom purple

Results and discussions

Specific pore volume

Mean ca. 0.52 cm³/g

Remarks on results incl. tables

Total pore volume (0.0018-100µm) was 0.52ml/g and average pore size was 88 nm.

4.33 Pour density

Endpoint study record: Pour density.001

Administrative Data

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

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	Survey on the current domestic and foreign risk assessment and the information on risk of nanomaterials	METI Survey report	JFE Tecno-Research				

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: JIS K 6219-2 (2006)	

Method

continuous drop

Test materials

Test material Identity

Identifier	Identity
	nanom purple

Any other information on materials and methods incl. tables

The principal material was poured into a cylinder of known volume from a height not more than 50mm without settling.

Results and discussions

Pour Density

Mean ca. 0.83 g/cm³

4.34 Photocatalytic activity

Endpoint study record: Photocatalytic activity.001

Administrative Data

Purpose flag key study

Study result type experimental result

Reliability 1 (reliable without restriction)

Data source

Reference

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

Overall remarks, attachments

Remarks on results including tables and figures

No photocatalytic activity

Removal rate of acetaldehyde and conversion rate to carbon dioxide

Post UV radiation time (hrs)	Materials					
	Fullerenes		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%	≤5%	≥95%	90.1%	≤5%	≤5%

5. ENVIRONMENTAL FATE AND PATHWAYS

5.1 Stability

5.1.1 Phototransformation in air

5.1.2 Hydrolysis

5.1.3 Phototransformation in water

Endpoint study record: Phototransformation in water.001

Administrative Data

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	US EPA: Qu, X., Hwang, Y.S., Alvarez, P.J.J., Bouchard, D. Li, Q.	2010	UV Irradiation and Humic Acid Mediate Aggregation of Aqueous Fullerene (nC60) Nanoparticles						

Materials and methods

Principles of method if other than guideline

Methods: Natural organic matter (NOM), ubiquitous in aquatic environments and soils, is one of the key factors governing the environmental transport and fate of colloids. NOM can adsorb onto colloidal particles, alter their surface properties and consequently affect their transport patterns. The capability of adsorbed NOM to stabilize colloids such as nC60 is well recognized. The steric hindrance effect has been reported to be responsible for the enhanced nC60 stability in the presence of humic acid. Suwannee River humic acid (SRHA) standard (II) was used as the surrogate for aquatic NOM. SRHA powder (100 mg) was dissolved in 100 mL of DI water and stirred for 24 hours in the dark. The pH was adjusted to 8.5 with NaOH to aid in the dissolution of SRHA. An aqueous nC60 stock suspension was prepared by pulverizing C60 powder using agate mortar and pestle for 10 minutes, taking 150 mg of the pulverized materials and adding it to 200 mL of DI water and subsequently sonicating at an intensity of 100 W for 30 minutes. After sonication, the mixture was filtered through 2-um pore-size Millex AP membrane filters to remove large particles. The resulting suspension had a C60 concentration of 4.4 mg/L. Irradiation was carried out in a Luxchem LZC-4 V photoreactor. Because UVA is the primary component of UV light in the solar irradiation that can reach the earth surface, a light source in the UVA wavelength range was employed to mimic the UV fraction of the solar spectrum. Four 8-W black lamps (Hitachi FL8BL-B $\lambda = 350 \pm 50$ nm) provided about 2 mW/cm² of light intensity at the center of the reactor. In each experiment, 30 mL of the nC60 stock solution at an unadjusted pH of 6.3 was added to a 50-mL Erlenmeyer flask, sealed with

parafilm with vents, placed on the merry-go-round at the center of the photoreactor, and slowly rotated to ensure uniform exposure. Samples were withdrawn on days 1, 2, 3, and 7 and stored in dark at 4 degrees centigrade before testing. Particle size of the pristine and irradiated nC60 was determined by dynamic light scattering (DLS) using SEN 3600 Zetasizer Nano equipped with a monochromatic coherent He-Ne laser with a fixed wavelength of 633 nm. Particle electrophoretic mobility was measured by phase analysis light scattering (PALS) using the same equipment as above. Aggregation of pristine and irradiated nC60 suspensions in various solution conditions was characterized by monitoring the intensity of weighted average particle size as a function of time via time-resolved DLS measurement. For experiments without humic acid, 0.25 mL of the nC60 stock suspension, predetermined amount of the electrolyte stock solution, and DI water were introduced into a disposable polystyrene cuvette to create a sample with a total volume of 1 mL and nC60 concentration of 1.1 mg/L. NaCl and CaCl₂ concentrations for pristine C60 and 7-day UV- irradiated nC60 were altered to show the effect of ionic strength on aggregation and electrophoretic mobility.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8

Results and discussions

Details on results

Results: The number mean and Z-average hydrodynamic radii of the pristine nC60 suspension were 54.8 +/- 2.4 nm and 82.3 +/- 0.9 nm, respectively. Particles were highly negatively charged with an electrophoresis mobility of $-3.34 \pm 0.13 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ in 1 mM NaCl. After seven days of UV-A irradiation, the particle characteristics remained unchanged. Pristine nC60 nanoparticles were stable only at NaCl concentrations below 20 mM, with a critical coagulation concentration (CCC) of 84 mM. After 7 days of irradiation, the stability of nC60 in NaCl increased drastically with a CCC of 449 mM NaCl. C60 aggregation rate decreased with increasing irradiation time. This is attributed to the presence of oxygen-containing functional groups on the surface of the irradiated nC60 particles. Particle electrophoretic mobility became less negative with increasing NaCl concentration. The increased stability in the presence of SRHA is attributed to the steric hindrance effect of humic acid molecules adsorbed on the nC60 surface. With 10 mg/L SRHA, no aggregation of pristine nC60 was observed at NaCl concentrations up to 1.5 M NaCl within 20 minutes. This further demonstrates that steric hindrance rather than electrostatic repulsion is the main stabilizing mechanism in the presence of humic acid, since the surface charge should be effectively screened at such an elevated NaCl concentration. However, the effect on the stability of 7 day UV-A irradiated nC60 was much weaker. This is attributed to decreased adsorption of humic acid due to increased hydrophilicity of the nC60 surface after UV irradiation. UV irradiation reduced nC60 stability in CaCl₂ due to specific interaction of Ca²⁺ with the negatively charged functional groups on UV-irradiated nC60 surface and the consequent charge neutralization. By neutralizing surface charges of both UV-irradiated nC60 and humic acid as well as forming intermolecular bridges, Ca²⁺ facilitated humic acid

adsorption on UV-irradiated nC60, resulting in enhanced stability in the presence of humic acid. As demonstrated in this study, solar irradiation could play a critical role in the aggregation behavior of nC60 and consequently its environmental fate and transport. While the stability of the pristine nC60 is largely controlled by the humic acid adsorbed on the nC60 surface, humic acid adsorption is mediated by the oxygen-containing surface functionality on the UV-irradiated nC60 particles. On the other hand, the extent of nC60 photochemical transformation is strongly influenced by humic acid. In addition, specific interactions between cationic species such as Ca^{2+} and nC60 surface functionality resulting from UV irradiation or humic acid adsorption can be a dominating factor in nC60 aggregation kinetics. These findings suggest that a better quantitative understanding of the dynamics of nC60 surface chemistry change is critical to predicting fullerene transport and fate in the aqueous environment as well as risk assessment for carbon based nanomaterials.

Endpoint study record: Phototransformation in water.003

Administrative Data

Study result type experimental result

Reliability other:

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Kong, L., Tedrow, O., Chan, Y.F., Zepp, R.	2009	"Light-Initiated Transformations of Fullerenol in Aqueous Media" (Light initiated formation of hydroxylated C60)						

Materials and methods

Principles of method if other than guideline

Methods: The fullerenols used in this experiment are of the salt form and the acid-precipitated form. Hydroxylated C60 is significantly more hydrophilic than C60. Depending on the number of hydroxyl groups, which varies from 12 to 40, the aqueous solubility may achieve 58.9 mg/mL, nearly 10 orders of magnitude greater than the aqueous solubility of C60. Published cytotoxicity and photo-toxicity data of fullerenol show a wide variety of responses from living cells or bacteria. Past studies indicate that fullerenol is reactive under certain conditions in aqueous media. Fullerenol also forms clusters in aqueous media and cluster formation potentially could influence its reactivity. The fullerenol solutions used in this research were prepared by magnetically stirring the fullerenol salt or acid-precipitated form with Nanopure water over a minimum of 24 hours. An Atlas SunTest CPS solar simulator equipped with a 1 kW xenon arc lamp was used for simulated solar irradiations and a rotating turntable reactor was used for monochromatic irradiations. Simulated solar irradiation experiments were conducted using completely filled quartz tubes sealed with Teflon lined septum caps that were submersed in a thermostated water bath. Monochromatic irradiation experiments were conducted in 8 mL Pyrex glass tubes that each contained 5.00 mL of solution, including ferrioxalate actinometers. Irradiation occurred for three weeks. Dark controls were contained in corresponding quartz or glass tubes wrapped with aluminum foil, and kept at room temperature. Fullerenol concentrations were measured using a Dionex Ultimate 3000 high performance liquid chromatography system and/or a Perkin Elmer Lambda 35 UV/Vis spectrometer. Electronic absorption spectra were

measured using the Perkin Elmer Lambda 35 UV/Vis spectrophotometer. Particle size distribution and zeta potentials were determined using a Malvern Zetasizer Nano ZS. A Shimadzu TOC-5050A was used to measure dissolved inorganic carbon (DIC) in the irradiated and non-irradiated fullereneol solutions.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8 MER Corp Fullerenols C ₆₀ (OH) _x (ONa) _y , with $x + y = 24$, $y = 6-10$, purity >99% (Alternate Material), MER Corp Acid Precipitated form C ₆₀ (OH) ₂₄ (Alternate Material)

Results and discussions

Details on results

Results: Dissolved inorganic carbon was identified as a major photoproduct of fullereneol and the ratio of moles DIC produced to moles of fullereneol reacted reached 28 or approximately 47% of complete mineralization on extensive irradiation by simulated solar radiation. A complete identification of fullereneol photoproducts was not available. Results of this study provide the first evidence that a fullerene derivative can be extensively mineralized under environmental conditions. DIC photoproducts were compounds such as CO₂, bicarbonate, and carbonate. The direct photoreaction kinetics of fullereneol in dilute aqueous solution can be described by pH-dependent biexponential rate expressions. This photoreaction slowed by a factor of 2 in nitrogen-saturated water. The oxygen dependence is attributed to photo-induced electron or hydrogen atom transfer from fullereneol to oxygen to produce superoxide ions with quantum yields of 6.2×10^{-4} . Fullereneol can photosensitize the production of singlet oxygen (¹O₂) in dilute aqueous solution with quantum yields ranging from 0.10 in acidic water to 0.05 in neutral and basic solution. However, our results indicate that chemical reactions involving diffusive encounters between ¹O₂ or superoxide and fullereneol are too slow to significantly contribute to the fast component of fullereneol photoreaction in sunlight. The pH dependence of the direct and sensitized photoreactions is attributed to changes in intramolecular hemiketal formation in fullereneol. The results also indicate that reactions involving diffusion together of fullereneol with O₂·-/HO₂· or ¹O₂, do not make a significant contribution together of the fullereneol photoreaction. The results further indicate that photo-induced electron or H-atom transfer to oxygen from electronically excited fullereneol can account for our observation that the fullereneol photolysis rate is higher in the presence of oxygen.

Endpoint study record: Phototransformation in water.004 (Stability in water)

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Isaacson, C., Bouchard, D.	2010	Effects of Humic Acid and Sunlight on the Generation and Aggregation State of Aqu/C60 Nanoparticles						

Materials and methods

Principles of method if other than guideline

Methods: C60 fullerenes are known to form charged nanoscale aggregates when suspended in water, resulting in mg/L aqu/C60 (fullerenes full suspended in water) concentrations despite C60's very low estimated water solubility of 7.96 ng/L. Additionally, the size, morphology, surface charge and mass suspended are sensitive to ionic strength and pH (Ma and Bouchard 2008; Fortner et al 2005; Dhawan et al 2006; Brant et al 2005; Brant et al 2006; Deguchi 2001). Previous studies indicate that the presence of humic substances increases the mass of aqu/C60 suspended while decreasing the size of the nanoparticles formed (Li et al 2009; Xie et al 2008; Terashima and Nagao 2007). Suwanee River Humic Acid was purchased from the International Humic Substances Society. Sunlight exposures were conducted between 9am and 5pm starting in August 2009 in Athens, GA. Sunlight-exposed and dark control aqu/C60 nanoparticles were generated by adding 100 mg of C60 powder to 400 ml of deionized (DI) water, 0.1, 1.0, 10 mg/L SRHA solutions adjusted to pH 7 and 100 uS/cm ionic strength (equivalent to 1mM NaCl) and Call's Creek water from a small stream near Athens, Ga (pH 7.9, conductivity 26 uS/cm, total dissolved solids 27 +/- 5.2 mg/L, and TOC 2.8 +/- 0.57 mg/L after filtration through a 0.45 um filter). Suspensions were capped and stirred in the presence of natural sunlight for 6 hours per day or in the absence of light covered in the laboratory. Sunlight had a total time average solar energy of 450 W/m² between wavelengths 300 and 940 nm. At each sampling event, suspension stirring was stopped and the suspensions were allowed to settle for 10 minutes, then a 10-mL aliquot of the suspension was removed from 1-2 cm below the suspension surface and immediately passed through a 0.45 um cellulose acetate filter. Quantification of C60 completed by liquid chromatography-atmospheric pressure photoionization-mass spectrometry (LC-APPI-MS). Size and zeta potential measurements made by DLS in batch mode on a Zetasizer NanoZs. Asymmetric flow field flow fractionation (AF4) method was coupled in flow-through mode to a DLS detector.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8 MER Corp C60 Powder

Results and discussions

Details on results

Results: Concentrations All dark treatments except the Call's Creek treatment had increased concentrations of aqu/C60 over the course of a 200 hour suspension time period. DI water, 0.1 mg/L and 1.0 mg/L humic acid treatment concentrations of aqu/C60 were not statistically significant from each other. However, the 10.0 mg/L treatment aqu/C60 concentration increased to a greater extent than the other dark treatments. This is likely due to increased steric stabilization of aqu/C60. Concentrations of aqu/C60 in the Call's Creek treatment did not increase with time and never exceeded 20 ug/L. This is possibly a consequence of divalent cation presence which can cause aggregation. All aqu/C60 suspensions formed under sunlight exhibited a trend where concentrations increased for the first 100 hours of suspension time and then maintained a constant concentration over the next 100 hours. DI water and Call's Creek treatments had the lowest concentrations which were not statistically significant from one another (having a pooled average of 150 +/- 18ug/L). Sunlight has a very significant role in dispersal of C60 in a variety of water types. In the Call's Creek water sunlight treatment concentrations were 17 times greater than the dark treatment concentrations. This difference was a factor of 8.1 greater in sunlight concentrations versus dark concentrations in the humic acid treatments, and smallest in the DI treatments with a 3.4 difference in sunlight concentrations versus dark concentrations. There appears to be an interaction between humic acid and sunlight: exposure to sunlight increased the aqu/C60 concentration in the humic acid treatments on average by a factor of 8.1 greater than the deionized water treatment. Size Batch measurements of all light and dark aqu/C60 treatments showed no time trend in the hydrodynamic diameter (Dh). However, after batch size measurements were pooled and averaged over the suspension time course, both light and dark suspensions generated in Call's Creek and DI water gave aqu/C60 hydrodynamic diameters much larger than any of the humic acid treatments. AF4 analyses indicate that aqu/C60 generated in the presence of sunlight varied in size and polydispersity depending on the background solution chemistry. Most of the aqu/C60 in DI water ranged in Dh from 140 to 300 nm. Aqu/C60 aggregates generated in 1.0 mg/L humic acid ranged in Dh from 120 to 300 nm and similar trends were observed for aqu/C60 generated in 0.1 and 10 mg/L humic acid. Unlike the skewed mass distribution size profile, the aqu/C60 mass was equally distributed about a mean intensity weighted Dh of 175 nm. Aqu/C60 generated in Call's Creek water ranged from 200 to 300 nm in Dh which is significantly larger than the DI or humic acid solution results. Zeta Potential For dark exposures, no time trend was observed for any of the treatment solutions. Over the time course, Call's Creek were consistently less negative than the other suspension, at -19 +/- 4.5 mV. The humic acid and DI water suspension zeta potentials were themselves not significantly different from each other with a pooled zeta potential of -44 +/- 3.1 mV. The zeta potentials of the aqu/C60 suspensions generated under sunlight in DI water and all humic acid treatments became more negative over the course of 150 hours and then remained constant for the next 50 hours. Aqu/C60 generated in Call's Creek water did not show this time trend and remained significantly less negatively charged than all other aqu/C60 sunlight treatments. This study demonstrates that sunlight and humic acid have significant effects on the concentration, size and zeta potential of the aqu/C60. Sunlight is clearly an important determinant of the mass of aqu/C60 available in the water column since sunlight increased the suspended mass of aqu/C60 in water from a surface stream by over an order of magnitude. The presence of humic acid decreased the size of aqu/C60 relative to the deionized water and Call's Creek suspensions, which has implications for toxicology and transport studies where particle size is an important variable. Finally, the utility of combining AF4-size classes was demonstrated, providing a new tool for higher resolution nanoparticle sizing under environmentally representative conditions.

Endpoint study record: Mer C60 TG316 Phototransformation in water.005**Administrative Data****Purpose flag** supporting study**Study result type** experimental study planned**Study period**

2010-2011

Reliability 4 (not assignable)

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. Standard USEPA or OECD harmonized guidelines (with modifications) were used where available.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Kong L.and R.G. Zepp,	2012	Production and Consumption of Reactive Oxygen Species by Fullerenes	Environmental Toxicology and Chemistry, 31(1): 136-143					

Materials and methods**GLP compliance**

no

Test materials**Test material identity**

Identifier	Identity
CAS number	99685-96-8 MER Corp. C60

Details on test material

C60 (99.9% pure) and fullerenol (acid-precipitated form) were purchased from MER Corp., Tucson AZ. Chemicals used for investigating the ROS were reagent-grade furfuryl alcohol (FFA), hydrogen peroxide (30%, w/w), and superoxide dismutase (SOD; 2,680 units/mg) from Sigma-Aldrich which were used as received.. Reagent-grade p-hydroxyphenylacetic acid (POHPAA) from Aldrich was purified by repeated recrystallization from ethanol. Sodium molybdate (99.5%) was purchased from Ventron, Alfa Division. Water purified with a Barnstead Nanopure D-7331 system was used in the experiments.

Study design**Details on test conditions**

Photolysis samples were prepared using pure water or pH buffered (4-9) pure water with low ionic strength (< 19 mM) to minimize aqu/nC60 and fullerenol aggregation during photolysis. To prepare aqueous C60 clusters (nC60), Aqueous suspensions of C60 were prepared by mixing ground C60 powder (400 mg) with Nanopure water (1 L) on a magnetic stirrer for at least four weeks. The stirring was stopped, and the

suspension was allowed to sit for 24 h prior to subsequent filtration (0.75-micron glass fiber filters). The filtrate was collected for use. A stock suspension of fulleranol was prepared by adding fulleranol powder into Nanopure water and mixing for 24 h. Working suspensions of fulleranol were obtained by dilutions of aliquots from this stock suspension. The aqueous suspensions of C60 and fulleranol were adjusted to neutral pH using 0.05M pH 7.0 phosphate buffer prior to use. An Atlas SunTest CPS solar simulator equipped with a 1 kW xenon arc lamp was used for simulated solar irradiations, and a rotating turntable reactor (a merry-go-round reactor (MGRR)) was used for monochromatic irradiations. The temperature was maintained at 22 deg C (± 1 °C using a NESLAB recirculating water bath. Glass filters (a combination of Corning 0-52 and 7-37 glass filters) were used to isolate the 366 nm radiation from a Hanovia mercury vapor lamp in the MGRR. Simulated solar irradiation experiments were conducted using completely filled quartz tubes (24 mL) sealed with Teflon-lined septum caps that were submersed in a thermostated water bath. The incident irradiance at the tube surface, summed from 290 to 700 nm, was 0.0065 W cm⁻². Monochromatic irradiation experiments were conducted in 8 mL Pyrex glass tubes that each contained 5.00 mL of solution. Particle size distributions and zeta potentials were determined using a Malvern Zetasizer Nano-ZS. Photoreaction quantum yield studies were performed in a rotating turntable reactor [i.e., a merry-go-round reactor (MGRR)], within which monochromatic light at 366 or 436 nm was isolated from a Hanovia mercury vapor lamp by a combination of Corning 0-52 and 7-37 glass filters or of Corning 3-73 and 5-58 glass filters, respectively. All samples were prepared in triplicate. Dark control samples were prepared in identical glass tubes wrapped with aluminum foil. Details for methods used to investigate photoproduction rates and quantum yields of singlet oxygen, superoxide-hydroperoxyl radicals, and hydrogen peroxide by nC60 and fulleranol are presented. Details also are presented for investigating and quantifying rate constants for reactions of nC60 and fulleranol with singlet oxygen (generated by photochemical and thermal techniques), superoxide/hydroperoxyl radicals, and hydroxyl radicals (generated by photochemical degradation of nitrate ions)

Any other information on materials and methods incl. tables

OECD GUIDELINES FOR THE TESTING OF CHEMICALS 316: Phototransformation of Chemicals in Water ? Direct Photolysis Photolysis samples were prepared using pure water or pH buffered (4-9) pure water with low ionic strength (< 19 mM) to minimize nC60 aggregation during photolysis. Because nC60 suspensions both absorb and scatter light the absorption and scattering coefficients for the nC60 suspension were measured using a Shimadzu UV-2450 spectrophotometer equipped with an ISR-2200 integrating sphere attachment (60 mm in i.d. and with BaSO₄ inside coating). A Perkin-Elmer Lambda 35 UV/visible spectrophotometer was used to measure the UV-visible absorbance of the fulleranol systems. Photolysis experiments were performed using the solar simulator or monochromatic light in the merry-go-round photoreactor. The light intensity was determined using a ferrioxalate actinometer and the quantum yields at these wavelengths were determined using the kinetic, spectral (fraction absorbed), and ferrioxalate actinometry data.

Results and discussions

Details on results

Techniques for testing the production and consumption of reactive oxygen species (ROS) (singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals) by aqueous suspensions of Buckminster fullerene (C60) and fulleranol are presented. Experimental protocols for the ROS studies as well as past more extensive reviews of ROS methods relevant to fullerene and other nanomaterials are included. Reactive oxygen species are one of the most important intermediates in chemical, photochemical, and biological processes that affect exposure concentrations of nanomaterials and related toxicity that is driven by these exposures. These methods will be useful in developing OECD protocols for indirect photoreactions and phototoxicity of nanomaterials. The tests conducted in this study showed that fulleranol has a higher

photoproduction efficiency of singlet oxygen and superoxide than aqueous suspensions of C60 aggregates (nC60), and this higher efficiency results in higher steady-state concentrations of these two ROS. Production of singlet oxygen by nC60 is accelerated by increasing oxygen concentration and in part is sensitized by fluorescent photoproducts that accumulate during irradiation. These results show that environmental conditions, including light exposure and oxygen concentration, have the potential to impact the generation of toxic ROS by fullerenes.

Overall remarks, attachments

Remarks on results including tables and figures

Findings of these tests show that both C60 and fulleranol can photosensitize the production of singlet oxygen, superoxide ions, and hydrogen peroxide under environmentally relevant conditions.

Aqueous suspensions of C60 (nC60) generally exhibited lower photoproduction efficiency of singlet oxygen and superoxide than fulleranol, most likely as a result of the different structures of the nC60 aggregates that cause efficient self-quenching of C60 triplet state precursors of ROS within the aggregates. This reduction in photochemical efficiency caused by the structure of C60 aggregates in water is partially offset by the production of hydroxylated, hydrophilic nC60 photoproducts that sensitize the production of singlet oxygen. This latter process results in increases in singlet oxygen photoproduction efficiencies by C60 aggregates in water with increasing light exposure. Both nC60 and fulleranol react very slowly with singlet oxygen. However, nC60 scavenges OH radicals at close to a diffusion-controlled rate and more than 12-fold more rapidly than fulleranol. Taken together, these results indicate that aggregation of nanomaterials such as fullerenes can strongly interact with their transformation and also photoproduction of toxic photoproducts. Sensitivity to light exposure, oxygen concentration, and other environmental conditions are likely to affect greatly the photosensitization of ROS and ROS scavenging properties of fullerenes and ultimately affect their roles in other physicochemical and biological processes.

Endpoint study record: MER C60 Fullerenes TG316

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability **incl.** 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. Standard USEPA or OECD harmonized guidelines (with modifications) were used where available.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Hou, W.-C., et al.	2010	Photochemistry of Aqueous C60 Clusters: Wavelength Dependency and Product Characterization.	Environmental Science & Technology, 2010, 44, 8121-8127					

Data access

data published

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
	OECD Guideline draft (Phototransformation of Chemicals in Water - Direct and Indirect Photolysis)	

GLP compliance

no

Test materials**Test material identity**

Identifier	Identity
CAS number	99685-96-8 MER Corp. C60

Details on test material

C60 was purchased from MER, corp. with a stated purity of 99.9%.

Study design**Details on test conditions**

To prepare aqueous C60 clusters (nC60), 80 mg C60 dry powder was magnetically stirred in pure water (1 L) for about 2 weeks prior to passing it through 0.7 and 0.45 µm membrane filters sequentially. The filtrate was collected for use. The size and zeta potential of the as-prepared nC60 were 150 nm and -45 mV, respectively. All solar irradiations occurred either on the roof of the Civil Engineering Building at Purdue University (West Lafayette, IN, 86° 55' W, 40° 26' N) or on a nearby farm field where sunlight intensity was recorded. In experiments that examined wavelength dependent effects, sample tubes were attached to the bottom of black boxes (5 cm in depth × 38 cm × 38 cm), on top of which 280 or 400 nm cutoff filters [Polycast UVT (280 nm cutoff) and UF-3 (400 nm cutoff), Spartech Corp., Clayton, MO] were clamped during exposure to sunlight. Photoreaction quantum yield studies were performed in a rotating turntable reactor [i.e., a merry-go-round reactor (MGRR)], within which monochromatic light at 366 or 436 nm was isolated from a Hanovia mercury vapor lamp by a combination of Corning 0-52 and 7-37 glass filters or of Corning 3-73 and 5-58 glass filters, respectively. All samples were prepared in triplicate. Dark control samples were prepared in identical glass tubes wrapped with aluminum foil.

Any other information on materials and methods incl. tables**OECD GUIDELINES FOR THE TESTING OF CHEMICALS 316:**

Phototransformation of Chemicals in Water ? Direct Photolysis Photolysis samples were prepared using pure water or pH buffered (4-9) pure water with low ionic strength (< 19 mM) to minimize nC60 aggregation during photolysis. Because nC60 suspensions both absorb and scatter light the absorption and scattering coefficients for the nC60 suspension were measured using a SHIMADZU UV-2450 spectrophotometer equipped with an ISR-2200 integrating sphere attachment (60 mm in i.d. and with BaSO₄ inside coating). Photolysis experiments were performed using natural sunlight (10am-4pm, May-

Oct.) or using monochromatic light (366 nm and 436 nm) in a merry-go-round photoreactor. The light intensity at 366 nm and 436 nm was determined using the ferrioxalate actinometer and the quantum yields at these wavelengths were determined using the kinetic, spectral (fraction absorbed), and ferrioxalate actinometry data. The photoproduct characterizations were performed on the C60 sample that was exposed to sunlight until C60 was not detectable (2 months). No product separation was performed due to the lack of reliable method for functionalized C60 products.

Results and discussions

Details on results

A general method is provided for investigating the photoreaction rates of nanoparticle suspensions in water. Aggregation of nanomaterials such as fullerenes often occurs in aqueous media. Light scattering by the aggregates changes affects the absorbance and phototransformation rates of such chemicals by altering the directional characteristics and pathlength of the light. A method that takes light scattering into account must be used to obtain reproducible, accurate results. Long-wavelength light ($\lambda \geq 400$ nm) isolated from sunlight, was shown to be important in both the phototransformation of nC60 and in the production of singlet oxygen. The significance of visible light in mediating the phototransformation of nC60 was supported by additional experiments with monochromatic light in which the apparent quantum yield at 436 nm ($\Phi_{436 \text{ nm}} = (2.08 \pm 0.08) \times 10^{-5}$) was comparable to that at 366 nm ($\Phi_{366 \text{ nm}} = (2.02 \pm 0.07) \times 10^{-5}$). LDI-TOF mass spectrometry indicated that most of the photoproducts formed after 947 h of irradiation in natural sunlight retain a 60-atom carbon structure. A combination of ^{13}C -NMR analysis of ^{13}C -enriched nC60, X-ray photoelectron spectroscopy and FTIR indicated that photoproducts have olefinic carbon atoms as well as a variety of oxygen-containing functional groups, including vinyl ether and carbonyl or carboxyl groups, whose presence destroys the native pi-electron system of C60.

Any other information on results incl. tables

Findings from this work suggest that C60 is unlikely to persist in sunlit aquatic environment (half life= ~ 1 day in noon sunlight). Sunlight exposure leads to hydroxylated and carboxylated C60 photoproducts with structure similar to fullerol (i.e., polyhydroxylated C60). Although the toxicity of the C60 photoproducts is currently unknown, previous studies have indicated that fullerol is less toxic to mammalian cells than pristine C60. A similar experimental approach may be applicable to other higher fullerenes (e.g., C60, C84, etc.). OECD 316 provides useful techniques for evaluating aqueous direct photolysis of chemicals in pure water, including nanomaterials. However there are several exceptions that apply to nanomaterials. First, the fact that many nanomaterials aggregate and "bundle" in water can create problems related to light scattering by the aggregates. OECD 316 applies to homogeneous systems in which molecules are interacting with the light, not aggregates. Examples of systems in which scattering has important effects on rates of photochemical processes are C60 suspensions in water (referred to as aqu/nC60 by some) and nano TiO₂ suspensions in water. One approach to dealing with light effects on aggregates is described in the Robust Summary for the Hou et al ES&T paper.

The other major effect not addressed by OECD 316 involves the effects of natural substances, in particular natural organic matter (NOM) on the light effects. OECD started to develop a test method for "indirect photolysis" of chemicals in natural waters but it has not been completed. NOM can retard light-induced processes by simply acting as a light screening agent. Techniques for evaluating light screening are well known and are mentioned in all of the summaries that I submitted. However, NOM also influences the efficiency of the photoreactions either by producing reactive transients that enhance the efficiency (i.e. it "sensitizes" photoreactions) or by scavenging transients (e.g. reactive oxygen species in oxygenated systems) that are produced by the chemical or by some other natural substance in the system such as nitrate or nitrite ions. The former effect is referred to as photosensitization or indirect photolysis and it can result

in transformation of chemicals that are photochemically inert in pure water. The scavenging effect is important in the case of fullerenes and TiO₂ for example. There is discussion of these various effects and methods for quantifying them in the ET&C paper by Kong and Zepp.

Endpoint study record: Mer corp C60 (OH) TG316

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability other:

Rationale for reliability incl. deficiencies The research was conducted using techniques that were defined by a quality assurance project plan in accordance with EPA QA/QC guidance. These techniques also were consistent with OECD Guideline 316 for conducting studies of phototransformation in water.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Kong, L et al	2009	Light-initiated transformations of fullereneol in aquatic environments.	Environ. Sci. Technol. 2009, 43, 9155-9160					

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline draft (Phototransformation of Chemicals in Water - Direct and Indirect Photolysis)	

GLP compliance

no

Test materials

Test material identity

Identifier	Identity
	Fullerenol

Details on test material

Fullerenol (acid-precipitated form, C₆₀ (OH)₂₄ and sodium salt form C₆₀ (OH)_x(ONa)_y, with x + y = 24, y= 6-10, purity >99%) (MER Tucson AZ)

Study design

Details on test conditions

Photolysis samples were prepared using pure water or pH buffered (4-9) pure water with low ionic strength (< 19 mM) to minimize fullerene aggregation during photolysis

Any other information on materials and methods incl. tables

Experiments were conducted using completely filled quartz tubes (24 mL) sealed with Teflon-lined septum caps that were submerged in a thermostated water bath. The incident irradiance at the tube surface, summed from 290 to 700 nm, was 0.0065 W cm⁻². Monochromatic irradiation experiments were conducted in 8 mL Pyrex glass tubes that each contained 5.00 mL of solution, including ferrioxalate actinometer. Electronic absorption spectra were measured using a Perkin-Elmer Lambda 35 UV/visible spectrophotometer. Particle size distributions and zeta potentials were determined using a Malvern Zetasizer Nano-ZS. Dissolved inorganic carbon (DIC) in the irradiated solutions and dark controls was measured using a Shimadzu TOC-5050A Carbon Analyzer. Each solution was measured in triplicate. Solutions of sodium bicarbonate and sodium carbonate were used as standards to calibrate the instrument. Photoproduction quantum yields of singlet oxygen by the fullerene at several pH values were measured using furfuryl alcohol as the singlet oxygen acceptor.

Results and discussions

Details on results

Dissolved inorganic carbon (DIC) was identified as a major photoproduct of fullerene, a hydroxylated C₆₀ molecule and the ratio of moles DIC produced to moles of fullerene reacted reached 28 or approximately 47% of complete mineralization on extensive irradiation by simulated solar radiation. The direct photoreaction kinetics of fullerene in dilute aqueous solution could be described by pH-dependent biexponential rate expressions. This photoreaction slowed by a factor of 2 in nitrogen-saturated water. The oxygen dependence was attributed to photoinduced electron or hydrogen atom transfer from fullerene to oxygen to produce superoxide ions with a quantum yield of 6.2×10^{-4} . The results also showed that fullerene can photosensitize the production of singlet oxygen in dilute aqueous solution with quantum yields ranging from 0.10 in acidic water to 0.05 in neutral and basic solution. However our results indicated that chemical reactions involving diffusive encounters between singlet oxygen or superoxide and fullerene are too slow to significantly contribute to the fast component of fullerene photoreaction in sunlight. The pH dependence of the direct and sensitized photoreactions is attributed to changes in intramolecular hemiketal formation in fullerene.

Any other information on results incl. tables

Hydroxylated fullerene derivatives are major degradation products of fullerenes as well as carbon nanotubes. The test results provided the first evidence that a fullerene derivative can be extensively mineralized under environmental conditions by direct photolysis. The results obtained from these tests were used to predictively model direct photolysis half-lives of fullerene under a variety of environmental conditions. The computed wavelength dependence of the specific sunlight absorption rate of fullerene indicated that absorption of both UV and visible wavelengths initiate photolysis with peak photoreactivity in the 330-430 nm range. Under simulated solar radiation the average half-life was 73 h of simulated solar irradiation in good agreement with the computer simulations. This corresponds to about 9.6 cloudless days of average sunlight exposure near the surface of water bodies during summer at latitude 40°N. OECD 316 provides useful techniques for evaluating aqueous direct photolysis of chemicals in pure water, including nanomaterials. However there are several exceptions that apply to nanomaterials. First, the fact that many nanomaterials aggregate and "bundle" in water can create problems related to light scattering by the

aggregates. OECD 316 applies to homogeneous systems in which molecules are interacting with the light, not aggregates. Examples of systems in which scattering has important effects on rates of photochemical processes are C60 suspensions in water (referred to as aqu/nC60 by some) and nano TiO₂ suspensions in water. One approach to dealing with light effects on aggregates is described in the Robust Summary for the Hou et al ES&T paper.

The other major effect not addressed by OECD 316 involves the effects of natural substances, in particular natural organic matter (NOM) on the light effects. OECD started to develop a test method for "indirect photolysis" of chemicals in natural waters but it has not been completed. NOM can retard light-induced processes by simply acting as a light screening agent. Techniques for evaluating light screening are well known and are mentioned in all of the summaries that I submitted. However, NOM also influences the efficiency of the photoreactions either by producing reactive transients that enhance the efficiency (i.e. it "sensitizes" photoreactions) or by scavenging transients (e.g. reactive oxygen species in oxygenated systems) that are produced by the chemical or by some other natural substance in the system such as nitrate or nitrite ions. The former effect is referred to as photosensitization or indirect photolysis and it can result in transformation of chemicals that are photochemically inert in pure water. The scavenging effect is important in the case of fullerenes and TiO₂ for example. There is discussion of these various effects and methods for quantifying them in the ET&C paper by Kong and Zepp.

5.2 Biodegradation

5.3 Bioaccumulation

5.3.1 Bioaccumulation: aquatic / sediment

Endpoint study record: Bioaccumulation: aquatic / sediment.001

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Patra, M., Ma, X., Isaacson, C., Bouchard, D., Poynton, H., Lazorchak, J., Roger, K.	2010	Changes in Agglomeration of Fullerenes During Ingestion and Excretion in <i>Thamnocephalus platyurus</i>						

Materials and methods

Principles of method if other than guideline

Due to the potential for C 60 nanoparticle aggregation, the exposure was conducted in low ionic strength media. In addition to extraction and analysis of the C 60 by GC, optical observations were used to analyze the extent of C 60 accumulation and thin layer TEM was used to investigate the aggregate structure of

particles in the gut.

GLP compliance

no data

Test materials

Test material identity

Identifier	Identity
CAS number	99685-96-8 MER Corp C60

Details on test material

For fullerene suspensions used for *T. platyurus* exposure, 100 mg of C60 or C70 was added to 400 ml double deionized water (DDI) water. Suspensions were stirred on a magnetic stir plate for 100 days. For particle size characterization prior to testing, suspensions were allowed to settle for 1 hour and the samples were taken 2 cm below the surface. The Z-average mean hydrodynamic diameters (D_h) of aqu/C60 and aqu/C70 aggregates prior to feeding measured by DLS were 517 +/- 21 nm and 656 +/- 39 nm, respectively. Similar to the results by DLS, analysis of the vortexed aqu/C60 suspensions prior to feeding by TEM showed aggregates that ranged from 200 to 500 nm in length. Zeta potentials for the aqu/C60 and aqu/C70 suspensions were -27.5 +/- 0.7 mV and -47.5 +/- 5.4 mV respectively.

Details on preparation of test solutions or sediment

The C60 (purity 99.9%) fullerenes were purchased from MER. The suspensions were stirred using a magnetic stir plate for approximately 100 d to form nanometer-sized fullerene aggregates that have been shown to be stable with respect to size and charge [7,13]. For particle size characterization prior to the exposure experiments, the suspensions were allowed to settle by gravity for 1h, and the suspensions were sampled at 2 cm below the surface. The C 60 suspension (aqu/C60) and was quantified using previously published methods. For mass quantification, aqu/C60 was dissolved into a toluene/methanol mixture (8:2 by volume) and quantified using liquid chromatography. Changes in fullerene aggregate size were examined by dynamic light scattering (DLS). Bouchard D, Ma X. 2008. Extraction and high performance liquid chromatographic analysis of C60, C70 and [6,6]-phenyl C61-butyric acid methyl ester in synthetic and natural waters. *Journal of Chromatography A* 120 (2008) 153-159.

Test organisms

Test organisms (species)

other: *Thamnocephalus platyurus*

Study design

Route of exposure

aqueous

Test type

static

Water media type

freshwater

Total exposure / uptake duration

1

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

The fullerene agglomerate burden was measured for two initial fullerene concentrations (3 and 6 mg/L) over 1 hour.

Any other information on materials and methods incl. tables

Thamnocephalus platyurus were hatched from cysts following the Rapidtox™ protocol. The cysts were hydrated with moderately hard reconstituted water (CaSO₄ 0.06 g/L, MgSO₄ 0.06 g/L, NaHCO₃ 0.096 g/L, KCl 0.004 g/L) and incubated at 25 °C under 4,000-lux illumination for 30 to 45 h. After hatching, the larval suspensions were transferred into DDI water and mixed (1:1) with gently vortexed fullerene suspensions and incubated for 1 h prior to addition of the colored indicator microspheres (5µm red polystyrene) into the fullerene ? organism mixtures. The organisms were exposed to the microspheres for 15 min prior to fixation with Lugol solution (final concentration, 1% KI, 0.5% I₂) and final observation.

Results and discussions***Details on results***

The uptake of aqu/C60 between 0 and 10 minutes was rapid for both concentrations. However, after the initial uptake period, the C60 concentration per unit mass of organism did not change significantly over the remaining 1 hour time period. Total aqu/C60 burdens were 2.7 ± 0.4 µg/mg and 6.8 ± 1.5 µg/mg wet weight for the 3 and 6 mg/L exposures, respectively.

Any other information on results incl. tables

The Z-average mean hydrodynamic diameters (D_h) of aqu/C60 and aqu/C70 aggregates prior to feeding measured by DLS were 517 ± 21 nm and 656 ± 39 nm, respectively. Similar to the results by DLS, analysis of the vortexed aqu/C60 suspensions prior to feeding by TEM showed aggregates that ranged from 200 to 500 nm in length. Zeta potentials for the aqu/C60 and aqu/C70 suspensions were -27.5 ± 0.7 mV and -47.5 ± 5.4 mV respectively.

After exposure of *T. platyurus* to aqu/C60 or aqu/C70 with no addition of indicator microspheres, the dark masses visible in the digestive tract also appeared to be segmented, indicative of the formation of excretion pellets. The observed percentage of organisms accumulating the dark masses was concentration dependent for both types of fullerenes. Further confirmation of aqu/C60 uptake was quantified by solvent extraction and analysis by HPLC-MS. The fullerene agglomerate burden was measured for two initial fullerene concentrations (3 and 6 mg/L) over 1 hour. The uptake of aqu/C60 between 0 and 10 minutes was rapid for both concentrations. However, after the initial uptake period, the C60 concentration per unit mass of organism did not change significantly over the remaining 1 hour time period. Total aqu/C60 burdens were 2.7 ± 0.4 µg/mg and 6.8 ± 1.5 µg/mg wet weight for the 3 and 6 mg/L exposures, respectively.

Transmission electron microscopy images of aqu/C60 exposed *T. platyurus* showed the formation in the gut of fullerene agglomerates 5-10 µm that were an order of magnitude larger than the suspended fullerene agglomerates. Upon excretion, the observed fullerene agglomerates were in the 10-70 µm size range and settled to the bottom of the incubation wells. The excreted C60 agglomerates were stored in DDI water at

room temperature and did not appear to disperse even after 6 months. On the other hand, exposure of the indicator spheres for several hours did not result in aggregates of these particles. Differences in the post excretion dispersion behavior of aqu/C60 and polystyrene microspheres may be due to their surface charge.

Applicant's summary and conclusion

Conclusions

The present study illustrates that anostacans such as *T. platyurus* show the potential to influence aqu/C60 fullerene agglomerate size. As a result, these organisms may facilitate the movement of fullerene agglomerates from the water column and into the sediment, potentially shifting exposure to benthic organisms in sediment.

5.3.2 Bioaccumulation: terrestrial

5.4 Transport and distribution

5.4.1 Adsorption / desorption

5.4.2 Henry's Law constant

5.4.3 Distribution modelling

5.4.4 Other distribution data

Endpoint study record: Other distribution data.001

Administrative Data

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	US EPA: Isaacson, C., Zhang, W., Powell, T., Ma, X., Bouchard, D.	2011	Temporal Changes in Aqu/C60 Physical-Chemical, Deposition, and Transport Characteristics in Aqueous Systems						

Materials and methods

Principles of method if other than guideline

Methods: Though the estimated water solubility of C60 fullerene is very low, C60 is readily suspended in water to form nanometer-sized aggregates at concentrations several orders of magnitude greater than its water solubility (Ma and Bouchard 2009; Fortner et al 2005; Isaacson and Bouchard 2010; Hwang and Li

2010). Recent studies have indicated that aqu/C60 may persist in aqueous phase for up to several months and that this dispersal is enhanced by humic acid and sunlight (Ma and Bouchard 2009; Isaacson and Bouchard 2010; Hwang and Li 2010; Hou et al 2010; Bouchard et al 2009). To create the aqu/C60 suspensions, 100 mg of C60 powder was added to 400 mL of deionized (DI) water and then stirred on magnetic stir plates under ambient laboratory lighting. Subsamples of aqu/C60 (fullerenes in a completely aqueous system) suspensions were collected from three different suspensions that were stirred for approximately 99, 309 and 1075 days (ie. short, intermediate and long intervals). Mixtures were allowed to free-settle for 24 hours and then 20-mL aliquots were collected 2 cm below the suspension surface. The aliquots were filtered through a prewashed 0.45 µm cellulose acetate filter. For the QCM and column transport studies which required larger sample volumes, multiple aliquots were collected as described and combined. Then the solution chemistry of the filtered aliquots was adjusted with 0.4 mM NaHCO₃ and 9.6 mM NaCl so that the solution pH was 7.8 and ionic strength was 10 mM. Quantification of aqu/C60 mass suspended in water was achieved by two methods: liquid-liquid extraction to toluene with salting-out by sodium chloride, or by dissolution of the aqueous suspension into a mixture of toluene and methanol. Concentrations of the C60 quantified by liquid chromatography atmospheric pressure photo-ionization mass spectrometry LC-APPI-MS. All C60 nuclear magnetic resonance (NMR) spectra were recorded on a Varian Inova 600 MHz spectrometer at 20 degrees centigrade with a 5-mm broadband probe. Aqu/C60 suspensions were prepared by stirring 3.1 mg of 20-30% C enriched C60 in 10 L of deionized water containing 10% H₂O. For characterization of the aqu/C60 suspensions, the UV/Vis light absorbance was recorded from 200 to 800 nm using a Lambda 35 spectrophotometer by Perkin Elmer. Size and zeta potential measurements were made by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS. Suspensions were also fractionated with asymmetric flow field flow fractionation (AF4) coupled with in-line (DLS). The deposition of aqu/C60 was monitored using a quartz microbalance with dissipation monitoring. The crystals used for the QCM-D studies were AT-cut quartz sensor crystals with a silica-coated surface. Column transport was assessed using angular high purity Iota quartz sand with 16% of the sand between 250 and 300 µm and 83% of the sand between 125 and 250 µm as a model porous medium. The quartz was sieved to select the particle 125-300 µm size grains, washed with deionized water, over-dried, and stored in a closed container prior to use. Pulse breakthrough experiments were conducted to characterize the transport properties of the short term, intermediate term and long term aqu/C60 suspensions in saturated sand under a steady-state pore water velocity.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
	MER Corp 20-30% C enriched C60

Results and discussions

Any other information on results incl. tables

Results: As suspension time increased, aqu/C₆₀ particle size shifted to a large size range as determined by AF4. Long term aqu/C₆₀ ranged in size from 240-410 nm in hydrodynamic diameter while short term

aqu/C₆₀ ranged from 220-360 nm in hydrodynamic diameter. Greater suspension time also correlated with a reduced distribution of aq/C₆₀ to toluene, likely due to surface polarization. The zeta potential did not change much over time.

Quartz crystal microbalance (QCM) deposition measurements indicate that the short term aq/C₆₀ was readily deposited on the SiO₂ surface while intermediate term aq/C₆₀ was less readily deposited and no deposition was observed for long term stirred aq/C₆₀. Atomic force microscopy (AFM) images show the size of the depositions. There was no trend in size across treatments. Most of the deposited aggregates were less than 150 nm. Selective deposition of the smallest aggregates is consistent with classic filtration theory which states that smaller particles have a greater propensity to be deposited, greater collector efficiency, due to their larger diffusion coefficient. However, increased surface polarity of aq/C₆₀ is likely a much greater contributor to the decreased deposition of the long-term stirred aq/C₆₀.

Transport of aq/C₆₀ occurred with no size-exclusion or retardation. This suggests that under steady state conditions the observed retention of the aq/C₆₀ resulted from the irreversible kinetic deposition, and not the equilibrium adsorption that would otherwise delay the breakthrough of the input pulses.

All three aq/C₆₀ suspensions were poorly retained in the saturated sand columns as the average mass recovered in the effluent ranged from 78 to 94%, indicating the aq/C₆₀ has the potential to travel significant distances upon entering into groundwater aquifers or river sediments.

The dynamic nature of aq/fullerene surface chemistry makes it problematic to estimate fullerene transport and fate in the environment. These results indicate that the analytical measurements using the popular toluene extraction technique are confounded by temporal changes in aq/C₆₀ extractability. One result of this effect is an underestimation of the actual aq/C₆₀ environmental concentrations due to lowered extraction efficiencies at longer fullerene-water contact times. Changes in aq/C₆₀ surface chemistry will also affect particle-particle and particle-surface interactions, likely resulting in increased suspension stability and decreased deposition on environmental surfaces. This emphasizes that environmental media such as water with which the nanoparticles interact could significantly influence the particle surface chemistry and consequently affect the fate and transport profiles of the environmental media.

Endpoint study record: Other distribution data.002

Administrative Data

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	US EPA: Zhang, W., Isaacson, C., Rattanaudompol, U-sa, Powell, T., Bouchard, D.	2012	Fullerene Nanoparticles Exhibit Greater Retention in Freshwater Sediment than in Model Porous Media						

Materials and methods

Principles of method if other than guideline

Methods: The fact that water-soluble C60 derivatives are readily dispersible in aqueous environments is expected. However, it was less expected when researchers found that hydrophobic C60 could form stable aqueous colloidal suspensions of nanoscale aggregates as a result of negative surface charge acquisition (Brant et al 2005; Bouchard et al 2009; Ma and Bouchard 2009). Aqu/C60 and water-soluble derivatives may consequently persist in the aqueous phase (Bouchard et al, 2009; Isaacson et al, 2011) and move large distances in the subsurface (Lecoanet et al, 2004; Cheng et al, 2005; Jaisi and Elimelech, 2009; Isaacson et al, 2011). Previous investigations on C60 transport have focused on model porous media that are less applicable to environmental systems involving natural soils, sediments or aquifer media. Bulk aqu/C60 suspensions were generated by stirring into DI water under ambient laboratory lighting for over 3 years, resulting in less hydrophobic aqu/C60 nanoparticles. Suspensions were allowed to settle for 24 hours; multiple 20-mL aliquots were collected approximately 2 cm beneath the surface and then passed through a pre-washed 0.45 μ m cellulose acetate syringe filter. Filtered aliquots adjusted to 0.4 mM NaHCO₃ + 9.6 mM NaCl and then re-filtered. Bulk aqu/C60-PTA suspensions were generated by stirring in DI water for only two months. Suspensions were then filtered through 0.45 μ m pre washed filters, and then directly diluted to form 10 mg/L C60-PTA suspensions of desired solution chemistries. Two model porous media, high purity Iota quartz (99.9% silica) and Ottawa sand (99.9% silica) were obtained from Unimin Corp. Freshwater sediment was collected from Call's creek near Athens, GA. All samples were sieved to desired size fractions, thoroughly rinsed with DI water, oven dried at 42 degrees centigrade and stored in a closed container prior to use. Call's creek sediment differed from Iota quartz and Ottawa sand in its greater surface physical and chemical heterogeneity represented by its much greater extracable Al, organic carbon, BET surface area, and surface roughness, as well as the existence of other minerals including mica (biotite), plagioclase feldspar (albite), and potassium feldspar (orthoclase). Natural sands tend to have patchy coatings of metal oxides or hydroxides that induce positive surface charge at near neutral pH. UV/VIS absorption spectra of aqu/C60 and C60 PTA suspensions were recorded from 215 to 700 nm using a UV/VIS spectrometer. Size and electrophoretic mobility (EM) of fullerene and sand colloid suspensions were measured by a Malvern ZetaSizer Nano ZS using dynamic light scattering (DLS) technique. The different media were packed into 1.5 cm inner diameter and 7 cm long Omnifit glass columns. Pulse breakthrough experiments were conducted to characterize transport properties of aqu/C60 and C60 PTA suspensions in saturated and unsaturated media under steady-state flows. The majority of the experiments were conducted at pH 7.5-8.0 and ionic strength (IS) 10 mM with varying media type, fullerene type and water content. Additional experiments were performed at pH 4 and 10 in saturated sediment to study the pH dependent fullerene transport. Derjagui-Landau-Verwey-Overbeek (XDLVO) interaction energies as a sum of Lifshitz-van der Waals, electrical double layer, Lewis acid-base, and Born repulsion interactions were calculated for aqu/C60 and C60-PTA nanoparticles interacting with quartz and other surfaces.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
	MER Corp C60 Powder

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

Results: Aqu/C₆₀ suspensions consisted of aggregates with a mean size of 172-195 nm. The AF4 analyses indicate that C₆₀-PTA in DI water were much smaller than aqu/C₆₀. The AFM measurement suggested that C₆₀-PTA in DI water may be in its singlet or slightly aggregated forms. In the tested suspensions, C₆₀-PTA nanoparticles were more aggregated due to increased IS (10 mM) and the smaller size at pH 4 may be a result of a low IS (1.5 mM). Aqu/C₆₀, C₆₀-PTA and sand grains were negatively charged at most environmental pH as their isoelectric points are less than 3. The zeta potential of grain surfaces became less negative in the order of Iota quartz, Ottawa sand and sediment. Measured logarithmic phase partition coefficient (K_{tw}) values for aqu/C₆₀ and C₆₀PTA were significantly greater at pH 4 than at other pHs, again due to neutralization of surface charge groups as solution pH approaches the Isoelectric points (IEP) of the fullerenes (Holmes-Farley et al 1985, 1988).

The aqu/C₆₀ and C₆₀-PTA had the least retention in Iota quartz, and the greatest retention in the sediment at near neutral pH and 10 mM IS, correlating with the degree of grain surface chemical heterogeneity. Similar to other colloids, fullerene retention was greater in unsaturated media than in saturated media at the 90% confidence level with the exception of C₆₀-PTA in Iota quartz. In general, aqu/C₆₀ was more retained than C₆₀-PTA at near neutral pH, due to the difference in interaction energies as discussed in the next section. Aqu/C₆₀ and C₆₀-PTA both exhibited pH-dependent transport behaviors (a slight decrease in retention when solution pH was increased to 10 and a significant increase in retention when solution pH was lowered to 4 even at lower IS of 1.5 mM).

Surface roughness was elucidated as another important factor responsible for the greatest fullerene retention in the sediment. In accordance with the XDLVO energy calculations, C₆₀PTA was less retained than aqu/C₆₀ at near neutral pH due to its greater hydrophobicity measured by toluene-water partition coefficient as well as smaller particle sizes revealed by atomic force microscopy.

Fullerene retention exhibited a strong dependency on solution pH that could be explained partly by the pH dependent surface charge of fullerenes and grain surface and partly by increased hydrophobicity of C₆₀-PTA when solution pH approaches its isoelectric points. Finally, fullerene retention was enhanced in saturated media, implying that fullerenes may be more attenuated in the vadose zone than in groundwater. Because fullerene retention was greater in the tested river sediment than in model porous media, fullerene nanomaterials may be more attenuated in some types of soils, river sediments, or groundwater aquifer than in model porous media (e.g., clean glass beads or quartz sand). This study is limited to one sediment sample, and more investigations are needed for other heterogeneous environmental media. Also, the vadose zone may serve as an effective barrier to mitigate the movement of nanomaterials.

5.5 Environmental data

5.6 Additional information on environmental fate and behaviour

Endpoint study record: Additional information on environmental fate and behaviour.001

Administrative Data

Study result type experimental result

Reliability other:

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Bouchard, D., Ma, X.	2008	“Extraction and High-Performance Liquid Chromatographic Analysis of C60, C70 and [6,6]-phenyl C61-butyric acid methyl ester in Synthetic and Natural Waters” (Synthetic and Natural Waters: Quantitative Detection Method)						

Materials and methods

Principles of method if other than guideline

Methods: All double deionized (DDI) water was obtained from a Purelab Plus water system and had a resistivity $> 18 \text{ m}\Omega/\text{cm}$. The surface water used was collected from a well-oxygenated first-order stream in the Georgia Piedmont region and the ground water was collected from a surficial aquifer in the same region. The pH, conductivity ($\mu\text{S}/\text{cm}$), and dissolved organic carbon (DOC) content (mg/L) of the surface ground waters were 6.81 and 6.12, 36.5 and < 10 , and 1.64 and 0.97, respectively. Treatments were prepared in DDI water and at pHs of 4, 7, and 10 with ionic strength equivalent to 10 mM NaCl. Solutions with different pH were buffered with 0.01 M acetate (pH 4.00 $\pm$ 0.01), HEPES (pH 7.01 $\pm$ 0.04), or Tris (pH 10.03 $\pm$ 0.06). Suspensions were formed using a stirring technique which does not employ organic solvents and is more representative of environmental conditions. After 13 days of stirring, liquid/liquid extraction of 1-mL volumes of the fullerene suspensions was employed. Subsequent large volume liquid/liquid and solid phase extraction was also employed. To investigate effects of $\text{Mg}(\text{ClO}_4)_2$ concentration on recovery efficiency, fullerene suspensions that were prepared in DDI water were extracted and at 0, 10, 50 and 100 mM concentrations $\text{Mg}(\text{ClO}_4)_2$. Quantitative analyses of the fullerenes' extracts and standards were performed using a Dionex Ultimate 3000 HPLC system with UV/vis-DAD. Separation of C60, C70 and PCBM was performed on a 4.6 mm x 150 mm Cosmosil 5 μ and 20% acetonitrile by volume flowing at a rate of 1.3 mL/min.

GLP compliance

no data

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8 MER Corp C60 Fullerenes

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

Results: Using a 80/20 v/v toluene-acetonitrile mobile phase and a 4.6 mm x 150 mm Cosmosil 5 u PYE column, C₆₀, C₇₀ and PCBM were fully resolved. Selectivity factors (alpha) for C₆₀ relative to PCBM and C₇₀ relative to C₆₀ were 3.18 and 2.19, respectively. The best analytical wavelengths for the fullerenes were determined to be 330, 333, and 333 nm with log molar absorption coefficients (log epsilon) of 4.3, 4.82 and 4.60 for PCBM, C₆₀ and C₇₀, respectively. Extraction and quantification of all three fullerenes in aqueous suspensions over a range of pH 4-10 and ionic strengths were very good. Whole method quantification limits for ground and surface suspensions were 2.87, 2.48 and 6.54 ug/L for PCBM, C₆₀ and C₇₀, respectively.

This work provides the first quantitative method for extraction and analysis of a suite of fullerenes from synthetic and natural waters. The data indicate that a simple liquid/liquid extraction procedure is adequate to extract PCBM, C₆₀ and C₇₀ from aqueous suspensions in natural waters as well aqueous suspensions over a range of pH and ionic strengths, and that these analytes can be separated from background interferences and quantified using high performance liquid chromatographic analyses (HPLC)-UV. This liquid/liquid extraction procedure in combination with the short (7 min) HPLC run times are particularly amenable to studies requiring the processing of large numbers of samples. When lower levels of quantification are required, larger sample volumes used in conjunction with larger solvent extraction volumes and subsequent volume reduction under N₂ provide whole-method quantification limits amenable for use in environmental research and monitoring studies.

Endpoint study record: Additional information on environmental fate and behaviour.002

Administrative Data**Study result type** experimental result**Reliability** other:

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Bouchard, D. Ma, X., Isaacson, C.	2009	Colloidal Properties of Aqueous Fullerenes: Isoelectric Points and Aggregation of C60 and C60 Derivatives						

Materials and methods

Principles of method if other than guideline

Methods: The solubility of C60 in common solvents is low, and it has a tendency to crystallize during film formation which limits its use in the fabrication of organic photovoltaic systems and other applications. To increase its solubility in a range of solvents and to modify its electronic properties, C60 is often derivatized. A common derivative is [6,6]-phenyl-C61-butyric-acid-methyl-ester (PCBM). Zheng et al 2004 studied PCB-R (where R is an alkyl group) esters and observed that PCB-R solubility increased in organic solvents with increasing alkyl chain length and that the butyl ester (PCBB) possessed better photosensitivity than PCBM. Fullerene aqueous suspensions were prepared in double deionized (DDI) water by mixing 100 mg of fullerene into 400 mg DDI and then stirring under ambient laboratory lighting for 5 months. C60 powder was ground with a glass mortar and pestle prior to mixing with DDI water due to its granular composition. The PCB-R fullerenes were added unaltered from the manufacturer. Suspensions were allowed to settle, 20 mL aliquots were obtained 1-2 cm below suspension surface, filtered through 0.45 µm cellulose acetate filter, and then aliquots were combined to form 100 mL working suspension volumes. Mass quantification of the fullerene aqueous suspensions was based on toluene extraction with HPLC-DAD (Bouchard and Ma, 2008). Aggregate sizes were measured using dynamic light scattering (DLS) with a ZetaSizer Nano ZS. Zeta potentials of the suspensions were also measured using a ZetaSizer Nano ZS instrument which employs phase analysis light scattering (PALS) to measure the electrophoretic mobility of charged particles. For pH titrations, titrants used were 0.50-1.0 M HCL, 0.1-0.4 M NaOH, and 0.01-0.05 M HCL. Fullerene aggregate size and zeta potential according to the various titrations were determined using the methods outlined above. Fullerene aggregation kinetics was determined by time-resolved DLS to measure fullerene aggregate intensity-averaged hydrodynamic diameter change with time and as a function of background solution ionic strength. In these experiments, 600 µL of aqueous fullerene suspension was pipetted into a quartz cuvette, and an additional 600 µL aliquot of either DDI or of a stock NaCl solution was added to yield a specific NaCl and fullerene concentration. The final background solutions included DDI and solutions with NaCl concentrations ranging from 25 to 1,000 mM NaCl. Measurements were conducted over various time periods ranging from 25 to 300 minutes.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8 Mer Corp C60 Powder

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

Results: The 190-800 nm UV-Scans for C₆₀ and the PCB-R fullerenes in 70/30 v/v toluene-acetonitrile are nearly identical. The best analytical wavelengths were determined at 330 nm for PCB-R fullerenes and 333 nm for C₆₀. Transmission electron microscopy (TEM) images indicate that the population of aqueous suspensions of n-C₆₀ aggregates (aqu/fullerenes) is poly-dispersed and characterized by both irregular and spherical structures. A recurring image was that of smaller, usually spherical particles oriented at the surface of larger particles. All aqu/fullerenes had zeta potentials less than -50 mV, isoelectric points (pI) were less than 1.0, and hydrodynamic diameter did not change significantly over the experimental time period indicating they were kinetically stable.

The effects of pH on aggregate charge and size were similar for all four aqu/fullerenes. Titration plots indicate that the zeta potentials for these aqu/fullerenes are relatively stable over the pH range of 4-9. Near pH 4, however, the zeta potential begins to increase with decreasing pH, and then increases rapidly between pH 2 and pH 1. As predicted by colloidal theory, aqu/fullerene aggregate size increases as zeta potential decreases in absolute magnitude.

The effect of NaCl concentration on aqu/fullerene aggregation kinetics was measured at a minimum of eight concentrations. Though differing in magnitude, the initial aggregation profiles of all the aqu/fullerenes studied are similar in form and dependent on background ionic strength. The critical coagulation concentration (CCC) values for the aqu/PCB-R fullerenes were significantly higher than that of aqu/C₆₀, indicating that the phenyl alkyl ester moieties of the aqu/PCB-R fullerenes were impeding the aggregation process.

All of the fullerenes studied formed kinetically stable aqueous suspensions which will increase their mobility and distribution in the environment relative to the hydrophobic molecular fullerene. With isoelectric points (pI) less than 1.0, aqu/C₆₀ and its aqu/PCB-R derivatives will have little acid/base functionality over the environmental pH range. The higher CCC values of the aqu/PCB-R fullerenes indicate greater colloidal stability than aqu/C₆₀, potentially resulting in increased residence time and bioavailability of these aqu/fullerenes in aqueous environments. These observations point to both the similarities and the differences in the aqueous behavior of aqu/C₆₀ and three of its derivatives.

Endpoint study record: Additional information on environmental fate and behaviour.003**Administrative Data**

Study result type experimental result

Reliability other:

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Ma, X., Bouchard, D.	2009	“Formation of Aqueous Suspensions of Fullerenes” (Effects of pH and Ionic Strength on Mass Aggregate Size and Zeta Potential)						

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

Methods: Seven treatments of aqueous suspensions were prepared in background solutions of double deionized water (DDI) water or at pH values of 4, 7, 10 with I equivalent to 10 mM NaCl, respectively. An amount of 100 mg of each material treatments were added to 400 mL of aqueous solution, the mixtures were stirred (time not given, no reference and no help in SI), but prior to sampling event the mixtures were allowed to settle for one hour. A 20 mL aliquot was collected 2 cm below the suspension surface and 20 mL of background solution was replenished after sampling. The samples collected were not representative of the total suspension volume, but rather, provided a consistent sampling point for very heterogeneous samples so that changes in suspension properties over time could be measured with acceptable precision. A 10 mL aliquot was filtered through a 0.45 um cellulose acetate filter (to allow comparison to prior studies which usually used filtered suspensions) while the other half-remained unfiltered (to represent an environmental sample collected in the field).The mass quantification was done through extraction into toluene and then analyzed by high performance liquid chromatographic analyses (HPLC)-UV. Zeta potentials of the suspensions were monitored using a ZetaSizer Nano ZS instrument. Changes in aggregate size were examined by dynamic light scattering (DLS) using the same instrument as above.

GLP compliance

no data

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8 MER Corp C60

Results and discussions

Any other information on results incl. tables

Results:

Aggregation Size

After 3 days of stirring, unfiltered aqu/C₆₀ (fullerenes in a completely aqueous system) suspensions had Z-average hydrodynamic diameters of 514 +/- 17 nm, 479 +/- 31 nm, and 535 +/- 9 nm in pH 7, pH 10 and DDI, respectively. These diameters remained constant over the period of the 111 day study; however pH 4 aqu/C₆₀ did not reach equilibrium of 767 +/- 63 nm until day 33. Particles are kinetically stable when they remain dispersed over a long period of time; they may collide by Brownian motion or shear flow, but do not stick together after the collision. The result is dispersed particles in media where size and zeta potential do not change over time. A limitation of DLS is the reliance on a single intensity averaged value, the Z-average diameter, for representation of the entire distribution of hydrodynamic diameters. The unfiltered aqu/C₇₀ suspension stabilized after day 16 to aggregate size of 725 +/- 52 nm.

For the filtered samples, aggregate size in most C₆₀ treatments was stable after day 8. Similar to the unfiltered samples, aqu/C₆₀ aggregate size in pH 4 filtered treatments did not stabilize as quickly as the other C₆₀ treatments. The data indicated that the 0.45 µm filters ? filtering occurred one hour after stirring the suspension ? removed particles larger than 450 nm and little post-filtration aggregation occurred. Pooling data from day 69-111 when aggregation size was stable, the Z-averages for the filtered C₆₀ samples were 191 +/- 23 nm, 171 +/- 13 nm, 165 +/- 4 nm and 164 +/- 7 nm for pH 4, 7, 10, and DDI, respectively. These were significantly smaller than the unfiltered treatments.

Background pH affected fullerene aggregate size in both unfiltered and filtered samples. In both cases, pH influenced aggregate size with largest aggregates forming in environments of pH 4. The increased proton activity may neutralize the negative charges at the aqu/C₆₀ surfaces. With less electrostatic repulsion, this resulted in van der Waals forces creating larger aggregates. It is generally thought that aqu/C₆₀ will quickly aggregate in natural waters, settle out of suspension and not pose an environmental risk (Lecoanet and Wiesner 2004; Espinasse et al 2007; Lyon et al 2005). However, the results from this study indicated that aqu/C₆₀ will form aggregates in water at common in natural waters (Pankow 1991) that are stable over a long period of time.

Mass

Unfiltered aqu/C₆₀ treatments reached stable concentrations after about day 26, and there was an inverse relationship between the mass of the fullerenes and the solution pH. As pH increased from 4 to 7 to 10 at the end of the 111 day experiment, the unfiltered aqu/C₆₀ mass concentration decreased from 107 to 84 to 43 mg/L. The mass in the aqu/C₇₀ unfiltered suspensions increased at a much slower rate than aqu/C₆₀. At the end of the 111-day sampling period the mass of the aqu/C₇₀ in DDI was only 35% that of the DDI treatment for aqu/C₆₀. Clearly, C₇₀, which has been the object of much less environmental study than C₆₀, forms aqueous suspensions far more slowly than C₆₀.

For most of the data, the mass in the filtered samples was generally < 1% of the mass in the unfiltered samples. The highest observed filtered concentration was 2.6 mg/L for aqu/C₆₀ at pH 10 which is consistent with previous observations that C₆₀ dispersed in water at a higher rate at basic pH values.

Zeta Potentials

In unfiltered treatments, with the exception of aqu/C₆₀ at pH 4, the zeta potentials were quite steady. The zeta potential for aqu/C₆₀ at pH 4 started to stabilize on day 26 concurrent with the stabilized size measurement. By the end of the experiment, all of the absolute zeta potentials were greater than 30 mV.

Particles with absolute potentials above 30 mV form stable colloidal suspensions due to strong electrostatic repulsions. Absolute zeta potentials were increased with increasing pH.

Zeta potentials for filtered samples were much more variable and a trend of decreasing zeta potentials over time was observed in all filtered treatments. However, by the end of the study, the potentials for the filtered and unfiltered had converged and were not significantly different.

Isoelectric Point (pI)

Results show that zeta potential increased as pH decreased for aqu/C₆₀. The zeta potential increased very rapidly as the isoelectric point (pI = 1.07) was approached. The pI of aqu/C₆₀ in DDI was significantly different from the C₆₀ treatments in pH 4, pH 7, and pH 10, indicating the effect of ionic strength on the formation of the aqu/C₆₀ suspensions. With isoelectric points approaching zero, the suspensions were generally stable at pH > 3.

The results from this study indicate that it is critical to state the condition under which the formation of aqueous fullerene suspensions occurs when employing such suspensions to evaluate environmental toxicity or fate and transport of fullerenes. For example, observed differences in pI were a function of the background solution parameters (pH, I) during fullerene suspension formation, not during pI measurement, thus indicating that solution characteristics during suspension formation have apparently irreversible effects on aggregate electrokinetic properties.

Endpoint study record: Additional information on environmental fate and behaviour.004

Administrative Data

Study result type experimental result

Reliability other:

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Xin, M., Wigington, B., Bouchard, D.	2010	Fullerene C60: Surface Energy and Interfacial Interactions in Aqueous Systems						

Materials and methods

Principles of method if other than guideline

Methods: Surface properties of Buckminster fullerene (C₆₀) and quartz surfaces were investigated. Application of the van Oss-Chaudhury-Good model and the Young-Dupre equation, the Lifshitz-van der Waals, acid-base, and the total surface energies of C₆₀ powder and quartz surfaces were calculated from contact angle measurements using the sessile drop technique.

Test materials

Test material identity

Identifier	Identity
CAS number	99685-96-8 MER Corp C60 Powder

Results and discussions

Any other information on results incl. tables

Results: C₆₀ powder surfaces are shown to have low-medium surface energy and the electromagnetic Lishitz-van der Waals component is the major component of the surface energy of C₆₀. In aqueous systems, hydrophobic attraction due to high cohesion of water is the driving force for C₆₀ aggregation. The high free energy of hydration indicates the high affinity of C₆₀ particles for water. Results of this study indicate that surface energy is an important physical parameter that should be considered as a basic characterization property of fullerene nanomaterials.

Endpoint study record: Additional information on environmental fate and behaviour.005

Administrative Data

Study result type experimental result

Reliability other:

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Isaacson, C., Bouchard, D.	2010	Asymmetric Flow Field Flow Fractionation of Aqueous C60 NPs with Size Determination by Dynamic Light Scattering and Quantification by Liquid Chromatography atmospheric Pressure Photo-Ionization Mass Spectrometry						

Materials and methods

Principles of method if other than guideline

Methods: Analytical methods need to be developed which can size separate materials in the nanometer range, while providing specific detection which allows for the differentiation between engineered and naturally occurring nanometer sized materials. Current methods to determine fullerene aggregate size distributions of aqueous C60 suspensions (aqu/C60) ? TEM, AFM and Light Scattering ? are not well suited for both large numbered samples and molecular composition determination. Asymmetric flow field flow fractionation (AF4) is an open channel size fractionation technique in which particles are separated based on differences in diffusion coefficients. AF4 is a versatile method for separating aggregates ranging in size from 1 to 500 nm in normal mode and under a range of elution solvent conditions. When employed

prior to size determination by light scattering, the occlusion of small aggregates by larger aggregates and the subsequent lack of resolution between aggregate populations is ameliorated. Aqueous fullerene suspensions were generated without organic solvents by high power sonication in deionized (DI) water using GENEQ ultrasonic processor delivering 300 W for 5 minutes, cooled for 5 minutes and then sonicated at the same power once more for another 5 minutes. The aqu/C₆₀ suspensions were then filtered with 0.45 µm cellulose acetate filters. The suspensions were filtered with 0.45 µm cellulose acetate filters. AF4 was coupled in flow-through mode to a dynamic light scattering (DLS) detector. Separation of C₆₀ was performed on Accela liquid chromatograph, Thermo Fisher. The Accela was connected to a Quantum Ultra triple quadrupole mass spectrometer to determine mass of each C₆₀ fraction. AF4 fractions were produced with an AF4 (Post Nova) with DI water eluent coupled in flow through mode to a DLS detector (ZetaSizer nano ZS). Characterization of the AF4 fractions was accomplished by aqu/C₆₀ extraction into toluene following destabilization of the colloidal suspension with NaCl and quantified with ¹³C₆₀ internal standard calibration by liquid chromatography atmospheric pressure photo-ionization mass spectrometry (LC-APPI-MS).

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8 Mer Corp C ₆₀ Powder

Results and discussions

Any other information on results incl. tables

Results: The initial un-fractionated aqua/C₆₀ suspension aggregates had a hydrodynamic diameter (D_h) of 130 +/- 1 nm, a zeta potential of -40 +/- 1 mV, polydispersity index of 0.16, and a C₆₀ mass concentration of 1.7 +/- 0.12 mg/L.

At a cross flow of 4mL/min, only 5.2 +/- 6.7% of the total aqua/C₆₀ mass had a D_h less than 80 nm, while 58 +/- 32% of the total aqua/C₆₀ mass had D_h between 80 and 150 nm and 14 +/- 9.2% of the total aqua/C₆₀ mass had D_h between 150 and 260 nm. With the optimal fractionation parameters, 77 +/- 5.8% of the aqua/C₆₀ mass eluted from the AF4 channel, indicating deposition on the AF4 membrane had occurred during fractionation; use of alternative membranes did not reduce deposition. Channel flow splitting increased detector response although channel split ratios greater than 80% of the channel flow led to decreased detector response. This is the first report on the use of AF4 for fractionating a colloidal suspension of aqua/C₆₀.

The polydisperse nature of aqua/C₆₀ suspensions with respect to particle size is problematic for size characterization of these suspensions, but is also problematic for colloidal transport studies, toxicity studies and other environmental studies with aqua/C₆₀ or other particulate species where size is an important variable. The AF4 methodologies described above provide for improved particle size characterization through fractionation followed by size determination by DLS and mass determination by mass spectrometry. In addition, since the fractionation method is non-destructive and can be performed under a wide range of background solution conditions, aqua/C₆₀ fractions with narrower size distributions can be

generated for further study in transport, toxicity or other studies.

Endpoint study record: Additional information on environmental fate and behaviour.006

Administrative Data

Study result type experimental result

Reliability other:

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	US EPA: Kikandi, S., Okello, V., Wang, Q., Sadik, O., Varner, K., Burns, S.	2011	Size-Exclusive Nanosensor for Quantitative Analysis of Fullerene C60						

Materials and methods

Principles of method if other than guideline

Methods: Conventional methods of assessing the properties and characteristics of raw nanomaterials focus on the size distribution and effects. They are, however, unsuitable for detection and quantification of complex environmental samples. The toxicity, detection and characterization of nanomaterials are dependent on factors such as functionalization, geometry and the type of nanomaterials. The first step to a better understanding of the fate and transport of fullerenes and other nanomaterials is to develop reliable metrology and analytical sensors. Nanosensors can be classified under two main categories: (i) Nanotechnology-enabled sensors or sensors that are themselves nanoscale or have components in the nanoscale, and (ii) Nanoproperty-quantifiable sensors. Engineered fullerenes C60 prepared via thermal reactions always exist as a mixture of C60, C70 and higher monologues. This study presents the development of Type II sensors for the isolation and quantitative analyses of engineered fullerene C60 while excluding other structurally similar fullerenes C70 and higher monologues. All microgravimetric quartz crystal microbalance (QCM) experiments were performed using a QCA 917-quartz crystal analyzer. The resonators consisted of gold thickness layer of 300 nm and a frequency resolution of 0.1 Hz at a sampling rate of 100 ms. These resonators were mounted onto a Teflon well-type electro-chemical cell then soaked with test solutions. Buffalo River Sediments soil samples were obtained from National Institute of Standards and Technology. The sensor fabrication started with the synthesis of β - or γ -cyclodextrin derivatives consisting of amino-functionalized derivatives of cyclodextrin designated as: β -CD-NH₂ (1) and γ -CD-NH₂ (2). Compounds 1 and 2 were immobilized separately on QCM via the DCC/NHS chemistry (Aluoch et al 2005). Amino-modified beta cyclodextrin (β -CD-NH₂) was confirmed by High Resolution Nuclear Magnetic Resonance as the host molecule to isolate the desired fullerene C60. This was subsequently assembled onto the surfaces of gold-coated quartz crystal microbalance (QCM) electrodes using N-Dicyclohexylcarbodiimide/ N-hydroxysuccinimide (DCC/NHS) surface immobilization chemistry to create a selective molecular configuration described as (Au)-S-(CH₂)₂-CONH- β -CD sensor. The mass change on the sensor configuration on the QCM was monitored for selective quantitative analysis of fullerene C60 from a C60/C70 mixture and soil samples. Nanosensor measurements were

carried out by mounting the (Au)-S-(CH₂)₂-CONH-β-CD sensor to the QCA 917-quartz crystal analyzer. In triplicates, exactly 200 μL of 0.14 mg/mL of C₆₀ or C₇₀ in toluene and control (toluene only) were placed on the modified QCM separately and the change in frequency was monitored over time. The changes were recorded as the immobilized β-CD on the QCM capturing the guest hydrophobic fullerene particles into their hydrophobic cavities. A decrease in frequency of 1 Hz corresponds to 1.34 ng and this was used to calculate the mass of the captured fullerenes. Time of flight-secondary ion mass spectrometry (ToF-SIMS) and field emission scanning electron microscope (FESEM) Zeiss Supra 55VP were used to characterize the surface chemistry during fabrication and testing experiments. An environmental application was carried out using 12 mg of C₆₀ and 10 mL of DI water which were added to 1 g of SRM soil samples. Then the soil samples were stirred at 1150 rpm under ambient conditions for 1 hour. One mL of anhydrous toluene was added and the resulting mixture was shaken for another 1 hour. C₆₀ was extracted into the organic phase. The extraction process was repeated 3 times with 1 mL of toluene. The extract was filtered through a 0.20 μm filter membrane and diluted to make 0.14 mg/mL C₆₀.

GLP compliance

no data

Test materials

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

no

Test material identity

Identifier	Identity
CAS number	99685-96-8 Sigma Aldrich: Fullerene-C ₆₀ , 98% (PN# 483036)

Results and discussions

Any other information on results incl. tables

Results: When the (Au)-S-(CH₂)₂-CONH-β-CD sensor was exposed to 0.14 mg/mL of C₆₀, C₇₀ and the control over time, the C₆₀ sensor showed a significant decrease in frequency while the C₇₀ and control remained relatively steady indicating no significant mass was absorbed.

About $\sim 10^{14}$ - 10^{16} C₆₀ particles/cm² were successfully quantified by QCM measurements. A continuous spike of 200 μL of 0.14 mg C₆₀/mL produced changes in frequency (- ? f) that varied exponentially with concentration.

FESEM and Time-of- Flight Secondary Ion Mass Spectrometry (ToF-SIMS) confirmed the validity of sensor surface chemistry before and after exposure to fullerene C₆₀. The utility of this sensor for spiked real- world soil samples has been demonstrated. Comparable sensitivity was obtained using both the soil and purified toluene samples.

In the environmental application, after capturing C₆₀ particles onto the sensor from pure and soil samples, the change in frequencies were 13251 and 9005 Hz, corresponding to $7.42 \cdot 10^{16}$ and $5.04 \cdot 10^{16}$ particles/cm², respectively.

This work demonstrates a new analytical concept that can be used to isolate and measure the number of engineered fullerene C₆₀ particles from pure and soil samples. Mixtures containing C₆₀/C₇₀ were isolated up to 20-fold C₇₀ concentration. Utilizing QCM's mass detection capabilities a Category II nanosensor concept was developed based on supra-molecular complexation of engineered fullerene nanoparticle onto

cyclodextrin. The sensor provides a size-exclusive approach for quantitative analysis of the nominated fullerenes. It is believed that this is the first Category II nanosensor reported for monitoring engineered fullerene C₆₀. The structural and morphological validity of the proposed sensor was confirmed using SIMS, NMR and SEM techniques, and the concept should be applicable to other fullerenes based on unique design of the sandwich configuration. The researchers have thus discovered a new practical measurement method for engineered fullerene C₆₀. Future work will focus on the application of the proposed sensor concept in wastewater samples, consumer products and food supplements (where lower detection limits will likely be needed).

6. ECOTOXICOLOGICAL INFORMATION

6.1 Aquatic toxicity

6.1.1 Short-term toxicity to fish

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

Administrative Data

Study result type experimental result
Reliability 2 (reliable with restrictions)
Rationale for reliability incl. deficiencies according to OECD TG

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Shinohara, N. et al.	2009	Is Lipid Peroxidation Induced by the Aqueous Suspension of Fullerene C ₆₀ Nanoparticles in the Brains of Cyprinus capio?	Environ. Sci. Technol. 2009, 43, 948-953					

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
CAS number	99685-96-8 Nanom Purple

Test organisms**Test organisms (species)**

Cyprinus carpio

Details on test organisms**TEST ORGANISM**

- Common name: C. carpio- Source: Niikura Fish Farm in Isehara, Kanagawa, Japan
- Age at study initiation (mean and range, SD): <1 year
- Length at study initiation (length definition, mean, range and SD): mean 85.5mm
- Weight at study initiation (mean and range, SD): mean 8.22g
- Feeding during test: not fed during the exposure test(48 h)

Study design**Test type**

static

Water media type

freshwater

Total exposure duration

48 h

Post exposure observation period

At the end of exposure period, the fish were anaesthetized.

Test conditions***Test temperature***

23.7-24.1°C

pH

7.3-7.7

Dissolved oxygen

6.0-8.3 mg/L

Nominal and measured concentrations

4.3-4.6 mg/L

Details on test conditions

TEST SYSTEM

- Test vessel: static aquarium with a cool water circulator
- Material, size, headspace, fill volume: glass, 56L
- Aeration: yes
- No. of organisms per vessel: 7
- No. of vessels per concentration (replicates): 7
- No. of vessels per vehicle control (replicates): 7

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: dechlorinated water
- Intervals of water quality measurement: the water was kept clear and colorless (concentration)

OTHER TEST CONDITIONS

- Photoperiod: 16:8 h light:dark cycle

Results and discussions

Details on results

- Behavioural abnormalities:
- Observations on body length and weight: control: 86 $\pm$ 6.3 mm, 8.4 $\pm$ 1.7 g, (brain) 0.14 $\pm$ 0.038g
exposure: 85 $\pm$ 3.7 mm, 8.1 $\pm$ 1.3 g, (brain) 0.13 $\pm$ 0.016 g
- Other biological observations:
- Mortality of control:
- Other adverse effects control: brain protein concentration: control: 6.0 $\pm$ 1.2 mg/mL exposure: 8.2 $\pm$ 1.2 mg/mL
brain LPO concentration: control: 0.082 $\pm$ 0.052 nmol/mg-protein exposure: 0.094 $\pm$ 0.063 nmol/mg-protein
- Abnormal responses:
- Any observations (e.g. precipitation) that might cause a difference between measured and nominal values:
- Effect concentrations exceeding solubility of substance in test medium: 85 + / - 3. 7

Overall remarks, attachments

Attached full study report

Illustration (picture/graph)

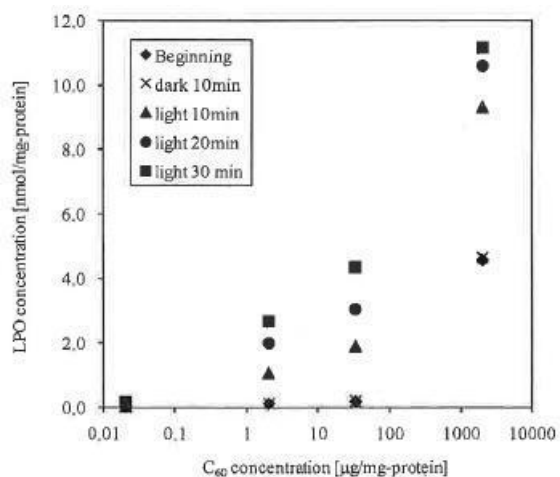


FIGURE 5. LPO concentrations against C₆₀ concentrations in the lipid sample extracted from brain tissue in the C₆₀ detection test.

Applicant's summary and conclusion

Conclusions

In the in vivo experiment, *C. carpio* was exposed to 4.5 mg/L nano C60 nanoparticle suspension for 48 h, following which the brain LPO concentration was measured. In the in vivo experiment, no fish died or exhibited abnormal symptoms during exposure. LPO assay of the *C. carpio* brain samples confirmed the absence of lipid peroxidation after exposure to 4.5 mg/L aqueous C60 nanoparticle suspension for 48 h. Additional LPO assay under irradiation showed that C60 did not reach the brain.

6.1.2 Long-term toxicity to fish

Endpoint study record: Long-term toxicity to fish.001

Administrative Data

Purpose flag	key study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	peer reviewed paper

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	T. W. K. FRASER et al.	2011	Dietary toxicity of single-walled carbon nanotubes and fullerenes (C60) in rainbow trout (<i>Oncorhynchus mykiss</i>)	Nanotoxicology, March 2011; 5(1); 98 ? 108					

Data access

data published

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

GLP compliance

no data

Test materials**Test material identity**

Identifier	Identity
CAS number	99685-96-8 C60 (SES Research)
	SWCNT (Cheap Tubes Inc.)

Details on test material

C60 (SES Research): Purity: 99.9% SWCNT (Cheap Tubes Inc.): 1.1 nm mean outside diameter, 5 ? 30 mm length minimum of 96.3% carbon (maximum impurities % were: Al 0.08, Cl 0.41, Co 2.91, and S 0.29)

Test organisms**Test organisms (species)***Oncorhynchus mykiss***Study design****Water media type**

freshwater

Post exposure observation period

two weeks

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

A 5 g / l stock solution for each CN was prepared in ultrapure (Millipore) water with 2% sodium dodecyl sulphate (SDS, 20 g / l) followed by sonication (bath type sonicator, 35 kHz frequency, Fisherbrand FB 11010, Germany) for 2.5 h, and stirring (Stuart type bench top stirrer set on a medium speed) for 3.5 ? 5.5 h. To ensure the method of food preparation was effective at dispersing CN among food pellets, the dietary marker yttrium oxide was added (15 g / l) to each stock solution. The stock solution was subsequently sonicated for 15 min and stirred for 4.5 h.

Juvenile female rainbow trout (n = 405) were obtained from Hatchlands Trout Farm, Rattery, and placed into a re-circulation system (with 20% water exchange / day) consisting of nine fiberglass aquaria (45 fish / tank) in a triplicate design (3 tanks / treatment, randomly assigned). Fish were held for 14 days prior to commencement of exposure and were fed a commercial trout food (see below) to satiation three times daily (photoperiod 12 h light: 12 h dark). During the experiment fish were exposed (six weeks) to either control (no CNs), SWCNTs (500 mg / kg), or C60 (500 mg / kg); 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 $\leq 2.8\%$ of their body weight (week 0 fish weight $17.7 \text{ g} \pm 0.6$; mean $\pm$ SEM, n = 45). Feeding rate was re-calculated daily based on mean weight of fish sampled during the experiment and a predicted growth rate of 2.5% / day, and feeding behaviour was monitored during feeding bouts to determine if food was being consumed. Fish were not fed during the 24 h prior to sample collection. 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 2800 spectrophotometer). Values were (mean $\pm$ SEM, n in brackets), pH, 6.72 ± 0.02 (86); temperature, $15.7 \pm 0.05^\circ\text{C}$ (85); percent oxygen saturation, $89.8 \pm 0.37\%$ (85); ammonia $< 0.01 \text{ mg / l}$ (42); nitrite, $0.44 \pm 0.03 \text{ mg / l}$ (47); nitrate, $3.37 \pm 0.31 \text{ mg / l}$ (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. Fish were randomly sampled prior to the commencement of the experiment (initial stock fish, n = 6) and then every two weeks during the experiment. 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 / treatment, n = 6 for initial fish).

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
6 wk						

Any other information on results incl. tables

Dietary exposure to carbon nanoparticles

There was no mortality or morbidity during the eight-week trial and there were no observed palatability differences among the diets. One fish (control) displayed abnormal swimming behaviour (chasing its tail)

beginning at week 4, and continued to behave abnormally until week 6 when it was sampled and subsequently excluded from analyses. Three fish (all C60 fish) were also excluded from analyses because they were below two standard deviations of the mean weight of all fish sampled at that specific time point (two at week 6, one at week 8) and were considered not to have fed or been exposed to the dietary treatment.

Growth and nutritional performance

Fish from all treatments maintained growth during the experiment (Figure 1) and no statistically significant (GLM, $p > 0.05$) differences were observed in mean final weight or food consumption (Figure 1); SGR (week 0 ? 6, mean $\pm$ SEM, (n); control 2.03 ± 0.23 (29), SWCNT 2.12 ± 0.10 (30), C60 2.19 ± 0.11 (28)), and FCR (week 0 ? 6, mean; control 1.12, SWCNT 1.04, C60 1.00) were also unaffected. Condition factor increased significantly (GLM, $p < 0.05$) over time in all treatments, but no differences were observed among treatments (week 6, mean $\pm$ SEM, (n); control 1.08 ± 0.02 (29), SWCNT 1.07 ± 0.02 (30), C60 1.11 ± 0.01 (28)). Carcass composition did not differ among treatments, however, significant time effects (associated with normal growth) were observed (GLM, $p < 0.05$), including decreases in moisture, protein, ash, and an increase in the amount of lipids (at week 6 mean values for all treatments were between 73 ? 74%, 58 ? 61%, 27 ? 31%, and 8 ? 9% for moisture, protein, lipids, and ash respectively).

Blood plasma analysis and tissue metal ion concentrations

Exposure to CNs had no statistically significant effect on plasma Na⁺, K⁺, plasma protein or glucose levels with mean values during the exposure remaining between 3.8 ? 4.7 mmol_l⁻¹, 142 ? 152 mmol_l⁻¹, 2.8 ? 3.1 g dl_l⁻¹, and 3.2 ? 4.8 mmol_l⁻¹, respectively, for all treatments. During the experiment significant decreases (GLM $p < 0.05$) in ion concentrations (Ca²⁺, Mg²⁺, Na⁺, K⁺) were observed in most tissues (intestine, liver, brain, spleen, and muscle), but these differences were not related to treatments (Table I). A significantly lower concentration of K⁺ and Mg²⁺ in intestine was observed in fish exposed to SWCNTs at week 2, but concentrations did not differ from those of control intestine in other weeks. Gill metals concentrations were within normal ranges and some minor changes occurred during the six-week experiment, but changes were not related to CNs exposure.

Na⁺K⁺-ATPase

Na⁺K⁺-ATPase activity in the gill, intestine, and brain were not affected by dietary exposure to CNs and levels did not change significantly during the experiment. The mean values ($\pm$ SEM) at week 6 for Na⁺K⁺-ATPase activity were; gill, 1.9 ± 0.3 , 1.6 ± 0.3 , 2.0 ± 0.3 ; intestine, 3.2 ± 0.6 , 2.7 ± 0.6 , 3.1 ± 1.0 ; brain, 23.5 ± 1.0 , 20.8 ± 1.2 , 23.5 ± 2.3 mmol Pi mg protein_l⁻¹ h_l⁻¹ for the control, SWCNTs, and C60, respectively.

TBARS and total glutathione

Brain TBARS was significantly higher in fish exposed to SWCNTs sampled at week 4 (Kruskal-Wallis, $p < 0.05$, Figure 2); however, this value did not differ significantly from brain TBARS measured in control fish at other time points. No other treatment effects on TBARS were observed in any tissue at any time. Over time across all treatments and control, TBARS (nmol mg_l⁻¹ protein) decreased in the gill (mean $\pm$ SEM; week 2, 11.49 ± 0.50 , 10.53 ± 0.76 , 10.81 ± 0.73 ; week 6, 9.29 ± 0.60 , 8.35 ± 1.15 , 9.08 ± 0.57 for control, SWCNT, and C60, respectively) and liver (mean $\pm$ SEM; week 2, 2.96 ± 0.36 , 2.90 ± 0.18 , 3.39 ± 0.36 ; week 6, 2.21 ± 0.26 , 2.01 ± 0.20 , 1.94 ± 0.17 for control, SWCNT, and C60, respectively) (GLM or Kruskal-Wallis, $p < 0.05$). No treatment or time effects were observed in TBARS in the intestine (mean $\pm$ SEM; week 6, 9.87 ± 1.33 , 8.23 ± 0.64 , 9.51 ± 2.03 for control, SWCNT and C60, respectively). During the experiment GSH levels (mmol g_l⁻¹) significantly decreased in the gill (mean $\pm$ SEM; week 2, 2.35 ± 0.13 , 2.39 ± 0.22 , 2.45 ± 0.17 ; week 6, 1.68 ± 0.25 , 1.88 ± 0.30 , 1.65 ± 0.25 for control, SWCNT, and C60, respectively), and brain over time (Figure 2), increased in the liver (mean $\pm$ SEM; week 2, 3.33 ± 0.34 , 4.00 ± 0.36 , 3.38 ± 0.40 ; week 6, 4.79 ± 0.14 , 4.62 ± 0.35 , 4.81 ± 0.42 for control, SWCNT, and C60,

respectively), whilst no time effects were observed in the intestine (mean $\pm$ SEM; week 6, 1.82 ± 0.41 , 1.45 ± 0.24 , 2.33 ± 0.67 for control, SWCNT, and C60, respectively); but no effects on GSH were related to CNs exposure.

Histopathology

The gills of fish from all treatments appeared normal with the usual background incidence of lesions expected from farmed fish including a few % of lamellae showing some clubbing of the tips, some oedema, or hyperplasia, and rare aneurism on individual lamellae. However, no treatment effects were observed in the percentage of secondary lamellae demonstrating these lesions (mean $\pm$ SEM, n in square brackets for total % of all lesions; control 4.4 ± 2.4 [6]; SWCNT, 4.7 ± 1.0 [6]; C60, 3.7 ± 1.2 [6]). The intestine had minor focal lesions consisting of a few necrotic cells that were not treatment related (n = 2 ? 6 / treatment, data not shown). There were no overt lesions observed in the brain, although occasional foci of condensed nuclei were observed in cells, from several areas of the brain, in all CN treatments (n = 6 / treatment, data not shown) and in control fish. A single focal lesion was observed in the liver of two C60 treated fish (n = 6 / treatment) and the lesion included pigmented macrophage aggregates, presence of inflammatory cells, necrotic hepatocytes, increased presence of fibrous connective tissue, and granulation tissue (Figure 3). The livers of fish from the control treatment (n = 6) showed normal histology at week 0 and week 6, except for two fish that had foci of pigmented macrophage aggregates. Pigmented macrophage aggregates were also observed in liver of two SWCNT-treated fish. The occurrence of vacuoles in hepatocytes decreased during the experiment in all treatments.

Overall remarks, attachments

Remarks on results including tables and figures

In conclusion, dietary exposure to either 500 mg / kg SWCNT or C60 had no effect on growth or haematology in rainbow trout over six weeks, and transient changes in TBARS were resolved during the exposure. Consequently, no particle shape effects could be discerned. However these data should be taken in context with other dietary contaminants of concern. The absence of effects on growth is not an indication of 'no effect' in dietary exposure studies (reviews, Clearwater et al. 2002; Handy et al. 2005). For example, dietary exposure to 500 mg / kg Cu in trout has no effect on growth and haematology, but profound effects on swimming speed, bioenergetics, and animal behaviour (e.g., Handy et al. 1999). Similar effects of CNs remain to be investigated. Furthermore we cannot exclude novel effects of CNs that remain undetected by the suite of endpoints used in this experiment, toxic effects that require longer exposures, or the novel effects of other CNs not investigated within this study.

Illustration (picture/graph)

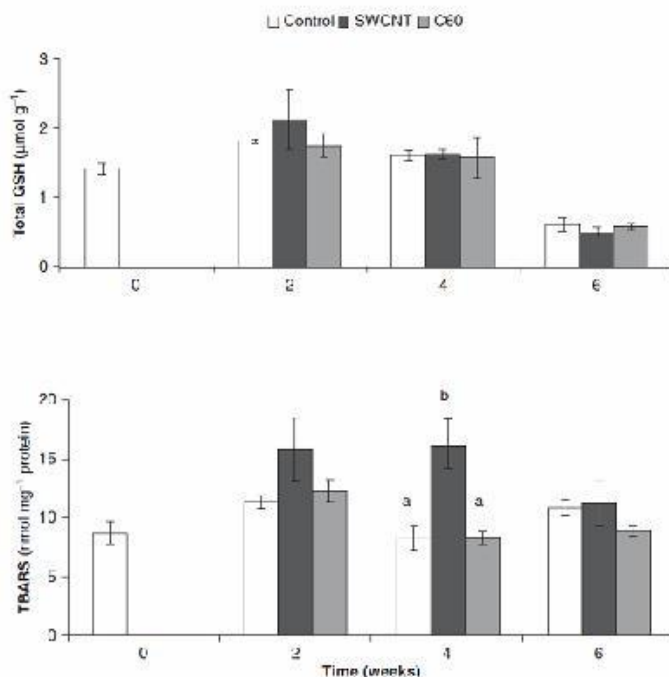


Fig. Total glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) in crude homogenates of brain from rainbow trout during six weeks' dietary exposure to control (normal food), 500 mg/kg single-walled carbon nanotubes (SWCNTs), or fullerenes (C60). Bars are means $\pm$ SEM ($n = 4-6$ fish / treatment time / point). Total GSH decreased in treatments and control during the experiment ($p < 0.05$), but no significant differences among treatments and the control were observed. No time effects were observed for TBARS in the brain, however, at week 4 TBARS was significantly higher in fish exposed to SWCNTs (One-way ANOVA at week 4, $p < 0.05$). Within week 4 data only, different letters indicate significant differences.

6.2 Sediment toxicity

6.3 Terrestrial toxicity

6.4 Biological effects monitoring

6.5 Biotransformation and kinetics

6.6 Additional ecotoxicological information