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DOSSIER ON SILICON DIOXIDE (NM 203) - PART 4 -

Series on the Safety of Manufactured Nanomaterials
No. 51

Disclaimer: This document has been modified to correct a typing error in the Foreword of the original document.

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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 51

DOSSIER ON SILICON DIOXIDE (NM 203)
- PART 4 -

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

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

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

No. 44, *Dossier on Gold nanoparticles (2015)*

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

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

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

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

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

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

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

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

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

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

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

This publication is available electronically, at no charge.

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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 testing[2]. 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 addressed[3] 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 sponsors[5] 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 synthetic amorphous silicon dioxide. This nanomaterial has been tested for a number of endpoints for: i) Nanomaterials Information / Identification; ii) Physical-Chemical Properties; iii) Environmental Fate; iv) Environmental Toxicology; v) Mammalian Toxicology; and vi) Material Safety. They have been analysed using OECD Guidelines for the Testing of Chemicals (TG)[8]. The data is presented in an IUCLID[9] style format and includes the protocols and methods used (see Preamble).

The European Commission and France co-led the Testing Programme on the Silicon dioxide. This included the determination of the tests that were appropriate, performing a number of tests, as well as coordinating tests and results obtained by other participating stakeholders from Belgium, Canada, Denmark, Japan, Korea and the Business and Industry Advisory Committee to the OECD (BIAC).

Due to the large amount of chemical substances used for the OECD Testing Programme on Silicone dioxide, the Dossier has been split into six parts:

- **Silicon Dioxide – NM 200**: ENV/JM/MONO(2015)14/PART1;
- **Silicon Dioxide – NM 201**: ENV/JM/MONO(2015)14/PART2;
- **Silicon Dioxide – NM 202**: ENV/JM/MONO(2015)14/PART3;
- **Silicon Dioxide – NM 203**: ENV/JM/MONO(2015)14/PART4;
- **Silicon Dioxide – NM 204**: ENV/JM/MONO(2015)14/PART5;
- **Silicon Dioxide – JP AIST data on SiO2 UFP-80 and NanoTek**: ENV/JM/MONO(2015)14/PART6.

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 Working Party on Manufactured Nanomaterials wish to thank the European Commission and France for co-leading the Testing Programme for Silicon Dioxide. They are specifically grateful to Kirsten Rasmussen from European Commission, as well as to Nathalie Thieriet and to Myriam Saihi from France. In addition, we appreciate the efforts made by other countries / organisations that participated in the Testing Programme, in particular Belgium, Canada, Denmark, Japan, and Korea, as well as the Business and Industry Advisory Committee to the OECD (BIAC).

TABLE OF CONTENTS

Name NM-203 silicon dioxide

Substance: NM-203 silicon dioxide

1. GENERAL INFORMATION

1.1 Identification

Substance identification

Chemical name NM-203 silicon dioxide

Reference substance

Reference substance silicon dioxide / dioxosilane / 7631-86-9

EC number	**EC name**
231-545-4	silicon dioxide
CAS number	**CAS name**
7631-86-9	
IUPAC name	
dioxosilane	

Type of substance

Composition mono constituent substance

Origin inorganic

1.2 Composition

1.3 Identifiers

1.4 Analytical information

Analytical information

Analytical methods and spectral data GC, HPLC and UV are not applicable.IR-Spectroscopy and ^{29}Si-NMR are well known analytical methods to identify and characterise SiO_2. With X-ray diffraction (XRD) the presence/absence and the content of crystalline fraction of SiO_2 can be determined.

Optical activity not applicable (no optical activity)

Results of analysis

Analysis type	XRD Analysis
Tested substance	NM-203 fumed (pyrogenic) SAS
Method used	X-ray diffraction
	XRD NM-203-1.pdf / 27.87 KB: SIAR

Remarks	See also chapter 4.25 crystalline phase (Evonik Industries AG 2013)
Analysis type	Infrared spectroscopy (DRIFT)
Tested substance	NM-203 fumed (pyrogenic) SAS
Method used	Infrared spectroscopy (IR-Spectroscopy)
	NM 203-7.pdf / 18.14 KB: SIAR
Remarks	Typical peaks for silanol groups of the particle surface, 3750 cm^{-1} (free), 3700 cm^{-1} (bridged), 3500 cm^{-1} (bridged with H_2O).

Analysis type	NMR
Tested substance	NM-203 fumed (pyrogenic) SAS
Method used	NMR Spectroscopy
	NM 203-8.pdf / 33.15 KB: SIAR
Remarks	Interpretation of the spectrum: The spectrum shows 3 overlapping signals in the chemical shift region of SiO_4- units, so called Q-groups (G. Engelhardt, D. Michel, High Resolution Solid State NMR of Silicates and Zeolithes, Wiley 1987). The following structural groups can be observed (see also deconvolution): -110.2 ppm notation: Q4 Description: SiO4 - unit, cross-linking group, every oxygen is connected to another silicon -100.1 ppm notation: Q3 Description: SiO4 - unit, branching group, three oxygens are connected to another silicon, one OH-group -91.1 ppm notation: Q2 Description: SiO4 - unit, middle group, two oxygens are connected to another silicon, two OH-groups. Table: ^{29}Si NMR (solid) signal assignment

Analysis type	ICP MS
Tested substance	Purity and impurities in NM-203 fumed (pyrogenic) SAS
Method used	e.g. DIN 66131, ISO 5794, ASTM C575 and D297
	NM 203-6.pdf / 28.06 KB

1.5 Joint submission

1.6 Sponsors

1.7 Suppliers

1.8 Recipients

1.9 Product and process oriented research and development

2. CLASSIFICATION AND LABELLING

2.1 GHS

2.2 DSD - DPD

3. MANUFACTURE, USE AND EXPOSURE

3.1 Technological process

3.2 Estimated quantities

3.3 Form in the supply chain

3.4 Identified uses and exposure scenarios

3.5 Uses advised against

3.6 Waste from production and use

3.7 Exposure estimates

3.8 Biocidal information

3.9 Application for authorisation of uses

4. PHYSICAL AND CHEMICAL PROPERTIES

4.1 Appearance

Endpoint study record: Appearance.001

Administrative Data

Purpose flag	key study ( ) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	other: laboratory observations	**Study period**	2009 ff
Reliability	1 (reliable without restriction)		

Data source

Reference

Reference type	other company data		
Author	Head of department ofr AT	**Year**	2009
Title	Particle Size of pyrogenic synthetic amorphous silica		
Bibliographic source	unpublished report		
Testing laboratory	Evonik Industries AG	**Report no.**	
Owner company	Evonik Industries AG		
Company study no.		**Report date**	

Data protection claimed

yes, but willing to share

Cross-reference to same study

See endpoint 4.5 Particle Size

Materials and methods

Test guideline/method

Qualifier no guideline required

Guideline

Deviations

Principles of method if other than guideline

During particle size analysis the test material is assessed and described.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial

NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Confidential details on test material

NM 203

Results and discussion

Physical state at 20ºC and 1013 hPa
solid

Form
powder

Colour
white

Odour
odourless

Substance type
inorganic

4.2 Melting point/freezing point

4.3 Boiling point

4.4 Density

Endpoint study record: Density/Tamped density.001

Administrative Data

Purpose flag key study ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Materials and methods

Type of method
other: graduated tapped cylinder

Principles of method if other than guideline
Tamped density (EN ISO 787/11) is measured to indicate the weight of the (bulk) product in powder form. Approximately 200 ml of material is subject to vibration (tapped 1,250 times) in a graduated cylinder. From the initial weight of the sample and the resulting volume, the tapped density is calculated and indicated in g/l.

Test materials

Test material equivalent to submission substance identity
yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Results and discussion

Density

Type tap density

Density ca. 44 g/L

Temp.

Overall remarks, attachments

Overall remarks

Tamped density of NM-203 is 44g/l

4.5 Particle size, size distribution

Endpoint study record: Particle size, size distribution of Agglomerates solid /air 001

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

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

Reliability 1 (reliable without restriction)

Data source

Reference

Reference type	study report		
Author	Head of Research Group	**Year**	2010
Title	Agglomerate-particle size under technical handling conditions		
Bibliographic source	USI TOX		
Testing laboratory	Technische Universität Dresden, Faculty of Mechanical Engineering, Institute of Process Engineering and Environmental Technology	**Report no.**	
Owner company	Evonik Industries AG		
Company study no.	USI TOX	**Report date**	2010-01-11

Data protection claimed

yes, but willing to share

Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-203 results regarding appearance are presented in endpoint 4.1

Materials and methods

Test guideline/method

Qualifier no guideline followed

Guideline

Deviations

Principles of method if other than guideline (including perfocmance, material limits, other limits)

Laser diffraction spectrometry (Test method in accordance to EN 481, ISO 9276-2)The He-Ne-Laser for optical spectroscopy was applied to determine the particle size distribution of NM-203. Therefore a focal distance of 1000 mm was employed for the measurement of the particles / agglomerates in a size range between 9 µm and 1750 µm. The Fraunhofer theory was employed for the evaluation of the detected signals.

Details on methods and data evaluation

Under these conditions characterized by a dry powder state, high solid concentration and low/no shearing of the product agglomerates are the relevant particles.

Data gathering

Instruments

Laser aerosol particle size spectrometer. The laser aerosol particle size spectrometer (LAP; Model LAP 321, TOPAS GmbH, Dresden, Germany) underlies the principle of light scattering. According to size of a particle passing the measuring volume scattered light occurs that is mapped on a photo detector situated in the dark field. Change of collected light at the photo detector is a measure of particle size and is subject to multi channel analysis. The counting results that are sorted into several channels according to their impulse height represent the basis for determining the number based particle size distribution by means of a calculation base and a calibration function. Sedimentation shaft GRADIS. The sedimentation shaft GRADIS is a dispersing system for dry powders, which is designed for the use in combination with the laser light spectrometer HELOS. The material is feed using an appropriate dosing system and falls onto two sloping planes (45°), which are located inside the sedimentation shaft. The whole drop high of the GRADIS is 630 mm. GRADIS in combination with HELOS. In addition to previous studies an oscillating conveyor was applied to feed the material into the sedimentation shaft.

Reproducibility

The particle size distributions were reproducibly determined. The median particle diameter weighted by volume amounts to 694 µm. The measured data of the three runs and the mean values of the median diameter, the arithmetic mean, the optical concentration, the standard deviation and the relative standard deviation are shown.

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

State of test material

dry bulk

Any other information on materials and methods incl. tables

	x50,3 [µm]	x [µm]	cont. [%]	rel. std dev. [µm]	[-]
1. Run	679.06	561.91	3.2	265.25	0.47
2. Run	697.20	584.54	1.9	264.46	0.45
3. Run	705.58	572.16	2.2	280.86	0.49
mean	693.95	572.87	2.4	270.19	0.47

Median diameters, arithmetic diameters, optical concentrations, standard deviations and relative standard deviations of NM-203 measured with the HELOS-GRADIS-System

Results and discussions

Mean diameter

≥ 694 µm

Particle size

Percentile D50

Mean ≥ 694 µm

St. dev. 2.4

Particle size distribution at different passages

No. #1

Size ≥ 679 µm

Distribution

No. #2

Size ≥ 697 µm

Distribution

No. #3

Size ≥ 705 µm

Distribution

Overall remarks, attachments

Overall remarks

The median particle diameter weighted by volume amounts to 694 µm.

Attached background material

Attached document NM 203_2.pdf / 14.2 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5

Remarks

Applicant's summary and conclusion

Conclusions

Under these conditions characterized by a dry powder state, high solid concentration and low/no shearing of the product agglomerates are the relevant particles.

Executive summary

The median particle diameter weighted by volume amounts to 694 µm.

Endpoint study record: Particle size, size distribution of Aggregates solid /air 001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	2009
Reliability	1 (reliable without restriction)		

Data source

Reference

Reference type	study report		
Author	Head of Research Group	**Year**	2010
Title	Aggregate-particle size under technical handling conditions		
Bibliographic source	USI TOX		
Testing laboratory	Technische Universität Dresden, Faculty of Mechanical Engineering, Institute of Process Engineering and Environmental Technology	**Report no.**	
Owner company	Evonik Industries AG		
Company study no.	USI TOX	**Report date**	2010-01-11

Data protection claimed

yes, but willing to share

Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-203

Materials and methods

Test guideline/method

Qualifier no guideline followed

Guideline

Deviations

Principles of method if other than guideline (including perfocmance, material limits, other limits)

Laser diffraction spectrometry (Test method in accordance to EN 481, ISO 9276-2)
The He-Ne-Laser for optical spectroscopy was applied to determine the particle size distribution of NM-

203. RBG in combination with HELOS

Details on methods and data evaluation

The RBG 1000 pressurizes the powder sample with a very high shear rate during the dispersion process. The intensity of the dispersion is considerably stronger than during the usual practical handling of powders. Due to this, the results using the RBG 1000 can be considered as “worst case”.

Data gathering

Instruments

Rotating brush generator (RBG 1000) in combination with HELOS

Reproducibility

The particle size distributions were reproducibly determined.

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

State of test material

dry bulk

Any other information on materials and methods incl. tables

Median diameters, optical concentrations and quantiles of NM-203 measured with the HELOS-RBG 1000-System

	x16,3 [µm]	x50,3 [µm]	x84,3 [µm]	copt. [%]
1. Run	3.73	7.18	17.04	1.50
2. Run	4.03	7.89	20.31	2.70
3. Run	4.12	7.76	15.24	4.50
mean	3.96	7.61	17.53	2.90

Detailed data of the three measurements are shown in the appendix.

Results and discussions

Mean diameter

≥ 7 µm

Particle size

Percentile D50

Mean ≥ 7.6 µm

St. dev.

Particle size distribution at different passages

No. #1

Size ≥ 7.2 µm

Distribution

No. #2

Size ≥ 7.9 µm

Distribution

No. #3

Size ≥ 7.8 µm

Distribution

Overall remarks, attachments

Overall remarks

The median particle diameter weighted by volume amounts to 7.6 µm.

Attached background material

Attached document NM-203-4.pdf / 44.15 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5

Remarks

Applicant's summary and conclusion

Conclusions

Under these conditions characterized by a dry powder state and high shearing of the product aggregates are the relevant particles. The intensity of the dispersion is considerably stronger than during the usual practical handling of powders. Due to this, the results using the RBG 1000 can be considered as “worst case”.

Executive summary

The median particle diameter weighted by volume amounts to 7.6 µm.

Endpoint study record: Particle size, size distribution of Aggregates solid/water 001

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Reliability 1 (reliable without restriction)

Data source

Reference

Reference type	study report		
Author	Head of Laboratory	**Year**	2009
Title	Determination of particle sizes in dispersion of water		
Bibliographic source	Department of AT		
Testing laboratory	AQURA GmbH	**Report no.**	A090016279
Owner company	Evonik Industries AG		
Company study no.	Department of AT	**Report date**	2013-09-10

Data protection claimed

yes, but willing to share

Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-203

Materials and methods

Test guideline/method

Qualifier no guideline followed

Guideline

Deviations

Methods

DLS

Principles of method if other than guideline (including perfocmance, material limits, other limits)

Method: Dynamic light scattering (DLS)Concentration: 60mg/lLiquid phase: WaterDispersion: 15min Ultrasound (Ultraschallstab Bandelin Sonopuls) Cycle 8, Power 100%

Details on methods and data evaluation

Aggregates are assemblies of primary particles which are fused together face-to-face in the form of chains or clusters. The aggregates are formed by the collision by primary particles during particle growth. Aggregates represent the smallest, stable, non-dispersible particle units of three-dimensional structure, with the size ranging from 100 – 700 nm for fumed synthetic amorphous silica in dispersion of water.

Data gathering

Instruments

Equipment: Malvern Nanosizer ZS

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	7631-86-9
Identifier	EC number
Identity	231-545-4
Identifier	IUPAC name
Identity	dioxosilane

State of test material

dry bulk

Results and discussions

Mean diameter

≥ 182 nm

Particle size

Percentile	D50
Mean	182 nm
St. dev.	

Applicant's summary and conclusion

Conclusions

Mean particle size diameter of aggregates in dispersion of water are > 180 nm.

Executive summary

Endpoint study record: Particle size, size distribution of Primary particles TEM 001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	experimental result
Reliability	1 (reliable without restriction)

Data source

Reference

Reference type	study report		
Author	Head of Laboratory	**Year**	2010
Title	Examination of primary particles via TEM		
Bibliographic source	Department of AT		
Testing laboratory	AQURA GmbH	**Report no.**	A100008425
Owner company	Evonik Industries AG		
Company study no.	Department of AT	**Report date**	2010-04-27

Data protection claimed

yes, but willing to share

Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-203

Materials and methods

Test guideline/method

Qualifier no guideline followed

Guideline other guideline: SOP

Deviations

Methods

TEM TGB

Principles of method if other than guideline (including perfocmance, material limits, other limits)

electron transmission microscopy (TEM)

Details on methods and data evaluation

Primary particles do not exist in isolation, they form aggregates and agglomerates. Aggregates are assemblies of primary particles which are fused together face-to-face in the form of chains or clusters. The aggregates are formed by the collision by primary particles during particle growth.

Data gathering

Instruments

Equipment: Hitachi 7500 transmission electron microscope Zeiss Libra 120

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

State of test material

dry bulk

Confidential details on test material

Sample preparation:10 mg SiO_2 (NM-203) are suspended in 1-2 ml of a H_2O/isopropyl alcohol mixture (1:2). The mixture is agitated for about 15 min. in an ultrasonic bath to separate the loosely coherent agglomerates into aggregates (the aggregates are formed from primary particles which are fused/grown together in the flame). One drop (10 µl) of the freshly agitated mixture containing the well-dispersed NM-203 is dosed (microsyringe) onto a TEM-copper-grid (400 mesh) which is covered by a thin film of carbonized (stabilized) Formvar (no holes).

Results and discussions

Mean diameter

≥ 10 nm

Remarks on results including tables and figures

Magnification up to: 200.000

Overall remarks, attachments

Overall remarks

Mean primary particle size calculated from BET specific surface area is scientifically not justified for non-monodispers, non-spherical, highly agglomerated particles.

Attached background material

Attached document NM-203-4.pdf / 15.76 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5

Remarks

Attached document NM 203-5.pdf / 390.07 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5

Remarks

Applicant's summary and conclusion

Executive summary

Endpoint study record: Particle size, size distribution by TEM

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	Keld Alstrup Jensen	**Year**	2012
Title	D4.2: Transmission electron microscopic characterisation of NANOGENOTOX nanomaterials. Key intrinsic physicochemical characteristics of NANOGENOTOX nanomaterials		
Bibliographic source	NANOGENOTOX Delivrable no. 5 Final Report		
Testing laboratory	CODA-CERVA (B), INRS (F), IMC-BAS (BG)	**Report no.**	D4.2
Owner company			
Company study no.		**Report date**	2012-05-01

Data access

other: Owner: NANOGENOTOX

Materials and methods

Test guideline/method

Qualifier equivalent or similar to

Guideline other guideline: NIST 960-1 Guideline

Deviations yes The general approach of the methodology is based on NIST 960-1 however it is not equivalent

Methods

TEM BF-TEM (Bright Field Transmission Electron Microscopy)

Principles of method if other than guideline (including perfocmance, material limits, other limits)

The general approach of the methodology is based on NIST 960-1 however it is not equivalent.

Details on methods and data evaluation

- To measure the characteristics of primary particles of a NM, the Feret Min and Feret Max were measured by CODA-CERVA following a systematic random sampling based on stereology at an appropriate magnification.
- The Feret Max and Feret Min were measured and the Feret Mean was calculated as the mean of Feret Min and Feret Max. The aspect ratio was calculated as the ratio of Feret Max and Feret Min. [Feret diameter is the distance between two tangents on opposite sides of the particle, parallel to some fixed direction. Feret max is the maxiumum projected lenght and Feret Minimum

the minimal one.]

- Micrographs were taken at 10 fixed positions determined by the microscope stage. On these micrographs a grid with a mesh of 100 nm by 100 nm was placed at random. The primary particles on each tenth intersection, counted from left to right were measured. When no particle was located at this intersection, the horizontal grid lines were followed until a primary particle was located on an intersection.
- The 'Detection module' of iTEM was used for threshold-based detection of the NM.
- The contrast and brightness of the micrographs were optimized, the involved particles were enclosed in a pre-defined frame or region of interest and thresholds were set to separate particles from the background based on their electron density and size. Particles consisting of less than fifty pixels and particles on the border of the frame were omitted from analysis. For each particle, twenty-three quantitative parameters, (described in Table 1-attachment), are measured and considered relevant for its characterization.
- Each particle detected in a micrograph was identified by a unique number, written in the overlay of the image. This allowed the selection of data of individual particles and the postanalysis deletion of erroneously detected particles.
- Artefacts were characterized by their morphology and a grey value lower than the mean grey value of the background plus three times its standard deviation. Particles fulfilling this criterion were identified and deleted automatically and particles with an unusual morphology, judged to be artefacts based on visual inspection on the micrographs, were omitted manually from analysis. (In addition to the micrograph related information, the intermediate and annotated images obtained during image analysis and the results and reports of these analyses were stored in the database, linked to the original micrograph)
- Descriptive statistics and histograms were calculated in Sigmaplot (Systat, Cosinus computing, Drunen, The Netherlands).
- The normality of the distributions of the measured parameters was tested with the Shapiro-Wilk and Kolmogorov-Smirnov tests, while the homogeneity of variances was tested with Spearman rank correlation test.
- Since these assumptions were not met, the non-parametric Kruskal-Wallis one way ANOVA was performed and data were compared pairwise with Dunn's Method to determine the micrograph and sample effects, and to determine the effect of sonication on the number of particles per grid area.
- The normality of the distributions and the homogeneity of variances were met for the mean values of the median mean diameter.
- A one way analysis of variance (ANOVA) was performed and data were compared pairwise with the Tukey test. The measured parameters were classified by principle component analysis usingo the SAS statistical software (SAS Institute Inc., Cary, NC, USA).
- Descriptive statistics and histograms were calculated in Sigmaplot (Systat, Cosinus Computing, Drunen, The Netherlands).

Used Protocols

1. Dispersion of the sample:NM sample was suspended in double distilled water at a concentration of 2.56 mg/ml and sonicated for 16 minutes using a Vibracell™ 75041 ultrasonifier (750 W, 20kHZ, Fisher Bioblock Scientific, Aalst, Belgium) equipped with a 13 mm horn (CV33) at 40 % amplitude. This setup resulted in an average horn power of about 26 W and a sample specific energy of 2530 ± 20 MJ/m³. During sonication the samples were cooled in icy water with ice to prevent excessive heating. After sonication, the samples were diluted to a concentration of 0.512 mg/ml.Details of used procedure can be found in the nanogenotox dispersion protocol file.
2. . Grid adjustment The charge of grid whas adjustes in order to allows for the attachment of the

negatively charged silica NM to the EM grid. Alcian blue pretreatment introduced positive charges on the surface of pioloform- and carbon-coated grids that tend to have a negative or neutral charge. (authors hand experience suggests that this approach is easier than the alternative based on glow discharging EM-grids with air to introduce negative charges and subsequent Mg2+ treatment, introducing positive charges). For TEM measurements the suspended NM was brought on pioloform- and carbon- coated, 400 mesh copper grids (Agar Scientific, Essex, England) that were pretreated with 1% Alcian blue (Fluka, Buchs, Schwitzerland).More details about the step by step procedures used for TEM analysis at Coda-Cerva can be found in protocols files

Used Protocols: attached files

Attached document	nanogenotox dispersion protocol.pdf / 777.29 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5
Remarks	Dispersion protocol
Attached document	Coating of dispersed NP in liquid on grids for TEM at CODA.doc / 45 KB (application/msword)
Remarks	This procedure aims to coat nanoparticles suspended in a liquid on EM-grids for TEM analysis
Attached document	Automatic_TEM_Coda_Cerva_SOP.doc / 43.5 KB (application/msword):): ENV/JM/MONO(2015)14/ANN5
Remarks	Protocol of automatic image analysis of nanoparticles at CODA-CERVA
Attached document	Semi-auto_TEM_Coda_Cerva_SOP.doc / 44 KB (application/msword): ENV/JM/MONO(2015)14/ANN5
Remarks	This protocol provides a step-by-step guide for semi-automatic detection and image analysis of nanoparticles at Coda Cerva. The protocol is conform with the ISO 13322-1:2004(E) “Particle size analysis"

Data gathering

Instruments

The samples were examined using a Tecnai Spirit microscope (FEI, Eindhoven, Netherlands) operation at 120Kvm at a spot size 3.

Calibration

Detals for calibration in Semi-automaitc and Automatic modes can be found in the protocol files.Basic Calibration:

• For each NM three independent samples were analyzed.

• Per sample, five micrographs were made with a 4*4 k Eagle CCD camera (FEI) at a magnification of 18500 times.

• For the given microscope and camera configuration, this magnification corresponds with a pixel size of 0.60 nm and a field of view of 2.45 μm by 2.45 μm. (This implies a lower particle size detection limit of approximately 6 nm, supporting on the criterion of Merkus (HG. Merkus, Particle Size Measurments, 1Edn. Pijnacker: Springer 2009) that large systematic size deviations can be avoided if the particle area is at least hundred pixels.)

• The field of view limits the upper size detection limit to 245 nm, one tenth of the image size as recommended in ISO 13322-1 (part 1, 2004)

Reproducibility

???

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

State of test material

other: fluffy powder

Overall remarks, attachments

Attached full study report

Draft_D4.2_TEM characterisation.pdf / 2.31 MB (application/pdf): ENV/JM/MONO(2015)14/ANN2

Applicant's summary and conclusion

Conclusions

Primary particle size measured by three institutions: 13 ± 6 nm, 4 5nm, 16 ± 3nm

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Size distribution and intensity averaged mean size of aggregates by DLS by CEA

Administrative Data

Purpose flag	( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	experimental result

Data source

Reference

Reference type	study report		
Author	K A Jensen	**Year**	2013
Title	D4.5: Surface charge, hydrodynamic size and size distributions by zetametry, dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS) in optimized aqueous suspensions for titanium and silicon dioxide.		
Bibliographic source			
Testing laboratory	CEA (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

End point: Homogeneity. Description of the method, instrument and sample preparation.

Materials and methods

Methods

DLS

Data gathering

Reproducibility

3 measurements

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

State of test material

other: fluffy powder in dispersion

Results and discussions

Remarks on results including tables and figures

Zeta size: 175.0 ± 7.4 nmIntensity distribution main peak (nm): 172.1 ± 15.5FWHM main peak (nm): 76.3 ± 13.4

Overall remarks, attachments

Attached full study report

D4.5_ZETA_DLS_SAXS_analysis.pdf / 3.6 MB (application/octet-stream): ENV/JM/MONO(2015)14/ANN5

Applicant's summary and conclusion

Cross-reference to other study

http://www.nanogenotox.eu/

4.6 N-octanol-water partition coefficient

Endpoint summary: N-octanol-water partition coefficient

Administrative Data

Short description of key information

Not applicable.NM-203 is not soluble in n-octanol.Note: This specific endpoint characteristics is inherent to the substance and is not linked to a specific lot.

4.7 Water solubility, hydrophilicity, dispersibility

Endpoint study record: Water solubility, hydrophilicity, dispersibility.001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	2012
Reliability	1 (reliable without restriction)		
Rationale for reliability	GLP Guideline study		

Data source

Reference

Reference type	study report		
Author	Study Director	**Year**	2012
Title	Determination of the solubility in water according to OECD 105 rsp. EU A.6		
Bibliographic source	USI TOX		
Testing laboratory	LAUS GmbH	**Report no.**	12060604G910
Owner company	Evonik Industries GmbH		
Company study no.	2013-0045-DGP	**Report date**	2013-04-02

Data protection claimed

yes, but willing to share

Materials and methods

Test guideline/method

Qualifier according to

Guideline OECD Guideline 105 (Water Solubility)

Deviations

Type of method

flask method examination at 20°C until plateau equlibration

Principles of method if other than guideline

based on prliminary experiments, the saturation equilibrium was achivable at 50 g/l.

Details on methods and data evaluation

The solubility of the test item in water was determined from the measured concentration of Silicon in the filtrated test solution of six individuel vessels using two different analytical methods. Solubility was performed at 20°C until plateau equlibration (no differences above 15% and no rising tendency in silicon concentration of the solution).

GLP compliance

yes (incl. certificate)

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

NM 203

Confidential details on test material

LOT 3151111314

Details on methods

first analytical Methode: AASsecond analytical Methode: UV/VIS 810 nm

Results and discussions

Water solubility

210.9 mg/L

Temp. 20 °C

pH > 4 — < 6

209.5 mg/L

Temp. 20 °C

pH > 4 — < 6

Details on results

c = 201.9 mg/l via AASc = 209.5 mg/l via UV VIS

Uncertainty according to GUM (Guide to the Expression of Uncertainty in Measurement)

Total Standard Deviation < 2.1 %

Applicant's summary and conclusion

Interpretation of results

moderately soluble (100-1000 mg/L)

Conclusions

NM 203 is soluble with 201 mg/l in water under described conditions.

4.8 Solubility in organic solvents / fat solubility

4.9 Surface tension

4.10 Flash point

4.11 Auto flammability

4.12 Flammability

Endpoint summary: Flammability

Administrative Data

Short description of key information

Method: VDI 2263-1Material does not catch fire. “Brennzahl” (BZ) 1Note: This specific endpoint characteristic is inherent to the substance and is not linked to a specific lot.

4.13 Explosiveness

Endpoint summary: Explosiveness

Administrative Data

Short description of key information

It is a matter of oxidizability if a substance can burn or not. Silica, chemically seen SiO_2, and silicates (or parts of them) are not oxidizable and, therefore, cannot burn. So, for silica and for silicates there is practically no risk of dust explosion.

Key parameter (optional)

Explosiveness non explosive

Discussion

However, during handling silica, even when emptying bags, the phenomenon of electrostatic discharges might be observed. Therefore, in the presence of combustible substances we recommend precautionary measures against static discharges, like earthing and occasionally inerting the gas atmosphere of the recipient vessel. More detailed information about this issue is given in our Technical Bulletin No. 62 „Synthetic Silica and Electrostatic Charges“.NM-203 is not dust-explosive. Note: This specific endpoint characteristic is inherent to the substance and is not linked to a specific lot.

4.14 Oxidising properties

4.15 Oxidation reduction potential

Endpoint summary: Oxidation reduction potential

Administrative Data

Short description of key information

Not applicable.Reason: Redox potential + IVNote: This specific endpoint characteristic is inherent to the substance and is not linked to a specific lot.

4.16 Stability in organic solvents and identity of relevant degradation products

4.17 Storage stability and reactivity towards container material

4.18 Stability: thermal, sunlight, metals

4.19 pH

4.20 Dissociation constant

4.21 Viscosity

4.22 Additional physico-chemical information

Endpoint study record: composition by DTA by ICM-BAS

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.3: Crystallite size, mineralogical and chemical purity of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	IMC-BAS (BG)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Endpoint investigated

other: composition by DTA

Details on methods and data evaluation

In DTA, the reference and the sample undergo identical thermal cycles; they are either heated orcooled

with the same rate. The temperature is measured for both sample and reference, and thedifference is calculated. Most transformations such as phase transitions, melting, crystallization,decomposition etc. are either endothermic or exothermic; that is they either require or releaseenergy. Thus when such a transformation takes place the temperature of the material will deviatefrom a reference. This is what is seen by DTA.IMC-BAS used a STA781 and DTA 675 from Stanton Redcroft for the differential thermal analysis (DTA). The heating rate was 10 ^{o}C/Min.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

Results and discussions

Results

the results were not found in the final report- to be checked

Overall remarks, attachments

Attached full study report

D4.3_MinChemComposition.pdf / 2.28 MB (application/octet-stream): ENV/JM/MONO(2015)14/ANN3

Applicant's summary and conclusion

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: composition by EDS by IMC-BAS

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.3: Crystallite size, mineralogical and chemical purity of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	IMC-BAS (BG)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: owner: NANOGENOTOX

Materials and methods

Endpoint investigated

other: composition by EDS

Details on methods and data evaluation

EDS is short for Energy-dispersive X-ray spectroscopy and may be available as an extra analytical tool in electron microscopes. The analysis is based on the fact that when hitting a material with charged particles, such as an electron beam, some of the electrons of the atoms in the matter under the beam will first be energized to higher orbital positions and then drop down to their appropriate energy level again during which X-rays are emitted. The emitted X-rays are characteristic for each element and have specific energetic wavelengths and energy patterns. Therefore an elemental composition can be quantified by analyzing the energy spectrum and intensities of the X-rays emitted during the analysis. EDS is mostly possible for Na and heavier elements. Lighter elements from Be and up may also be quantified depending detectors and instrumental configuration. Oxygen is normally not analysed by SEM EDS, but may be calculated by difference or by converting all elements to oxides. When calculated by difference, as done in this work, the sum of all elements adds up to 100 wt%. Measurements may be made as semiquantitative or quantitative analyses using either standardless/internal instrument standard values or calibrated concentration-intensity curves using arange of relevant metals, minerals and glass standards, respectively. In the present analysis, elements were reported as semi-quantitative results. Due to current quality of detectors and in-build standard references, such results are relatively reliable for major elements if the materials have sufficiently high thickness and low roughness. Samples were prepared by pelletizing a known amount of powder. The results are given in wt.% and parts per million (ppm) depending on the absolute concentrations in the sample materials.

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Results and discussions

Results

Na (ppm/wt%): 0; Al (ppm/wt%): 4300 ; Si (wt%): 46.32; S (ppm/wt%): 400; Ca(ppm/wt%): 0; O (wt%): 53.21

Remarks on results including tables and figures

no data on the instruments used.

Overall remarks, attachments

Attached full study report

D4.3_MinChemComposition.pdf / 2.28 MB (application/octet-stream): ENV/JM/MONO(2015)14/ANN3

Applicant's summary and conclusion

Conclusions

Sample only contain minor elemental impurities. The presence of calc-alkali elements, S and Al support the analyses (XRD) with occasional observation of Na sulfate and boehmite.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: composition by ICP_OES by CODA-CERVA

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.3: Crystallite size, mineralogical and chemical purity of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	CODA-CERVA (B)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Endpoint investigated

other: Elemental composition

Details on methods and data evaluation

All measurements were carried out with inductively coupled plasma-optical emission spectrometry (Varian 720-ES, Agilent Technologies), using the SemiQuant feature, which is designed to provide afast estimate of the concentration of non-calibrated compounds in samples. The samples werescreened for 68 elements (Figure 5-1) (Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu,Fe, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pd, Pr, Pt, Rb, Re, Rh,Ru, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn, Zr).Sample preparation: To bring the NM sample in solution, 0.1 g was weighed in a 50 ml DigiPREP HT tube (SCPSCIENCE) and 2 ml of concentrated HF was added. The mixture was heated overnight at 80°C in a DigiPREP MS (SCP SCIENCE). After cooling, the volume was made up to 10 ml with double distilled water.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

Results and discussions

Results

Impurities ranges found for NM-203 Impurities 0.005-0.01 %: Na (in vial 0244 only)

Overall remarks, attachments

Attached full study report

D4.3_MinChemComposition.pdf / 2.28 MB (application/octet-stream): ENV/JM/MONO(2015)14/ANN3

Applicant's summary and conclusion

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: composition by TGA by NRCWE

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2013

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	
Title	Deliverable 4.3: Crystallite size, mineralogical and chemical purity of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	NRCWE (DK)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: owner: NANOGENOTOX

Materials and methods

Endpoint investigated

other: mass lost by TGA

Details on methods and data evaluation

In a thermograviometric measurement a sample is heated in a gas (usually air, O_2 or N_2) and the weight of the sample is measured as a function of the temperature. The decomposition temperature and loss of mass may give information about the sample, e.g. water adsorbed to the surface of particles will evaporate around 100 °C, whereas most other associated or technically added organiccoatings will evaporate or combust at higher temperature. A decomposition in several steps will indicate a non-homogeneous sample containing several different types combustable compounds, which could in fact all be structurally different carbon nanotubes.Instruments:For the thermogravimetric analysis (TGA) NRCWE used a Mettler Toledo TGA/SDTA 851e and anoxygen atmosphere. The heating rate was 10 K/min and the same temperature rangefrom 25 °C to 1000 °C. The sample holders used for the TGA measurements were made of aluminaand had a volume of 70 µL or 150 µL.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

Any other information on materials and methods incl. tables

The SOP used for TGA analysis:
Thermogravimetric Analysis (TGA.) Renie Birkedal (NRCWE) based on NIST Recommended Practice Guide, Special Publication960-19
General description
TGA is short for thermogravimetric analysis. The principle is measuring sample weight as a function of temperature in a given atmosphere at a given heating rate. TGA is measured according to information wanted and material investigated. If information about evaporation is wanted heating in N_2 is recommended. If information about organic content is wanted heating in O_2 or air is recommended, as this will insure combustion of all organic material. In order to make sure e.g. all organic material is decomposed, it is recommended to run to 1000 °C. Materials and Chemicals:Powder (may be conditioned in a specific atmosphere and humidity conditions)
Laboratory weigh (scale)Apparatus for thermogravimetric analysis
Procedure:
Sample preparation:
♣ Weigh container.
♣ Fill container with material. Do not stamp it, as this may affect theevaporation/decomposition temperature.
♣ Weigh container and material.For inorganic powder materials a minimum of 10 mg should be used – if possible more. These samples are usually quite homogeneous and this is usually a representative fraction of the sample. At the same time these compounds often have a low density, and it is therefore difficult to measure a representative fraction in one or two measurements. The solution is many measurements and comparison of the data. Selection of heating rate. For inorganic materials only a minor fraction is expected to decompose, and a heating rate of 10°C/min is recommended. It is not assumed that there will be large weight losses for these materials, so this heating rate ensures a fast measurement and most likely still well defined weight losses. If the weight losses are not well defined a slower heating rate can be chosen.The NIST Recommended Practice Guide, Special Publication 960-19, Measurement Issues in Single Wall Carbon Nanotubes, recommends a heating rate of 5°C/min. This is chosen as a compromise between time and avoiding too much spontaneous combustion. For some carbon nanotubes 5°C/min is not slow enough to avoid spontaneous combustion. There is no spontaneous combustion with a heating rate of 2.5°C/min. The measurement time is very long, app 7 hours per measurement, but this is still recommended. In order to minimize measuring time it may be an option only to heat to 900°C or even lower.
Data treatment:
Compare TGA curve and curve for first derivative to find steps of weight loss. It is recommended to obtain several measurements to calculate the mean and standard deviation of the weight loss and the evaporation/decomposition temperatures. (the last is most easily found from the curve of the first derivative). The test of multiple samples also enables evaluation of sample homogeneity.

Results and discussions

Results

TGA measurements on the NM sample were performed once only as the quantities analyzed were sufficiently large to be representative, and the main purpose for these measurements has been to detect coating on the materials. NM203 did not react thermogravimetrically in the oxygen atmosphere suggesting presence of no organics.

Overall remarks, attachments

Attached full study report

D4.3_MinChemComposition.pdf / 2.28 MB (application/octet-stream): ENV/JM/MONO(2015)14/ANN3

Applicant's summary and conclusion

Conclusions

No mass loss observedPhase transtion detected at 324°C (DTA)

Cross-reference to other study

http://www.nanogenotox.eu/

4.23 Agglomeration/aggregation

Endpoint study record: Agglomeration/aggregation.001

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Reliability 1 (reliable without restriction)

Data source

Reference

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

Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-203

Materials and methods

Methods

DLS

Principles of method if other than guideline (including perfocmance, material limits, other limits)

Method: Dynamic light scattering (DLS)

Details on methods and data evaluation

Aggregates are assemblies of primary particles which are fused together face-to-face in the form of chains or clusters. The aggregates are formed by the collision by primary particles during particle growth. Aggregates represent the smallest, stable, non-dispersible particle units of three-dimensional structure, with the size ranging from 100 – 700 nm for fumed synthetic amorphous silica.

Data gathering

Instruments

Equipment: Malvern Nanosizer ZS

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

State of test material

dispersion

Any other information on materials and methods incl. tables

Concentration: 60mg/lLiquid phase: WaterDispersion: 15min Ultrasound (Ultraschallstab Bandelin Sonopuls) Cycle 8, Power 100%

Results and discussions

Agglomerate/aggregate diameter

Mean diameter

≥ 182 µm

Agglomerate/aggregate size

Percentile D50

Mean ≥ 182 µm

St. dev.

Overall remarks, attachments

Overall remarks

Aggregates represent the smallest, stable, non-dispersible particle units of three-dimensional structure, with the size ranging from 100 – 700 nm for NM-203.

Endpoint study record: Agglomeration/aggregation by DLS

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	D4.5: Surface charge, hydrodynamic size and size distributions by zetametry, dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS) in optimized aqueous suspensions for titanium and silicon dioxide.		
Bibliographic source			
Testing laboratory	CEA (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Methods

DLS

Data gathering

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Results and discussions

Agglomerate/aggregate diameter

Agglomerate/aggregate size

Percentile

Mean 175

St. dev. 7.4

Applicant's summary and conclusion

Conclusions

DLS Zeta aggregate size [nm]: 175±7.4

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Agglomeration/aggregation by SAXS

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	
Title	D4.5: Surface charge, hydrodynamic size and size distributions by zetametry, dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS) in optimized aqueous suspensions for titanium and silicon dioxide.		
Bibliographic source			
Testing laboratory	CEA (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Methods

other: SAXS

Principles of method if other than guideline (including perfocmance, material limits, other limits)

Small-Angle X-ray Scattering is a technique based on the interaction between X-rays and electrons to probe the structure of materials. The processed data is the number of X-ray scattered by a sample as a function of angular position of a detector

Details on methods and data evaluation

For Silica nanomaterials a sonication of 6.82 mg/mL NM suspension in ultrapure water is performed also at 40% amplitude for 20 min in ice-water cooling bath

Data gathering

Instruments

X-ray generator : Rigaku generator RUH3000 with copper rotating anode (λ= 1.54 Å), 3kW

♣ Home made optic pathways and sample holders (with two channel-cut Ge (111) crystals inBonse/Hart geometry for USAXS set up, cf Lambard (1992).

♣ Flux measurement for SAXS set up : pico amperemeter Keithley 615

♣ Flux measurement for USAXS set up : DonPhysik ionization chamber

♣ Detector for SAXS set up : 2D image plate detector MAR300

♣ Detector for USAXS set up: 1D high count rate CyberStar X200 associated to a scintillator/photomultiplier detector.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Applicant's summary and conclusion

Conclusions

agglomerate size could not be determined because the parameters could not be fitted by the model

Cross-reference to other study

http://www.nanogenotox.eu/

4.24 Crystalline phase

Endpoint study record: Crystalline phase.001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	2009
Reliability	1 (reliable without restriction)		

Data source

Reference

Reference type	study report		
Author	Head of laboratory	**Year**	2009
Title	Röntgenbeugungsuntersuchung		
Bibliographic source	Company Report USI-TOX		
Testing laboratory	AQura GmbH	**Report no.**	A090022571
Owner company	Evonik Industries AG		
Company study no.	2008-0175 DKP	**Report date**	2009-12-03

Data protection claimed

yes, but willing to share

Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-203

Materials and methods

Test guideline/method

Qualifier according to

Guideline other guideline: XRD Analysis

Deviations

Methods

x-ray diffraction (XRD)

Details on methods and data evaluation

Test Procedure: No sample treatment has been carried out prior to the measurements. A portion of the silica powder as received has just been applied to a standard sampler holder of the Diffractometer.

Used Protocols: attached files

Attached document phys chem endpoints NM-203 1 OECD.pdf / 27.87 KB (application/pdf): ENV/JM/MONO(2015)14/ANN2, ENV/JM/MONO(2015)14/ANN3

Remarks

Data gathering

Instruments

The measurements are conducted by means of a X´Pert Pro X –Ray Diffractometer (PANalytical) operating in Bragg Brentano 0-0geometry, and equipped with a copper cathode (wavelength of lambda= 1.54 Å (Cuka) and a X'Celerator. Diffractrograms have been collected at a temperature of 25°C in a range of 4°-74° 2-Theta range at step width of 0,017° and a step time of 80 sec.

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

State of test material

dry bulk

Overall remarks, attachments

Overall remarks

It can be concluded, the product is fully amorphous; no crystalline structure can be determined.

Applicant's summary and conclusion

Conclusions

NM-203 is fully amorphous; no crystalline structure can be determined.

Endpoint study record: Crystalline phase by XRD by NRCWE

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.3: Crystallite size, mineralogical and chemical purity of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	NRCWE(DK)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Methods

x-ray diffraction (XRD)

Principles of method if other than guideline

X-Ray Diffraction (XRD) analysis is based on the principle that crystalline materials diffract X-rays in a characteristic pattern, which is unique for each material. XRD can therefore be used to identify different polymorphs, such as typical TiO_2 polymorphs rutile, brookite and anatase.The width of the reflections can also give information about the size of the diffracting crystals (not necessarily the same as the particle size). XRD can be measured in different setups and different wavelengths are possible, but for standard measurements this is less important, as long as it is taken into account. Most databases are based on irradiation using Cu X-rays. The step length (if using Cu) is recommended to be 0.15. (Hill, 1986) All data presented in this report were recorded in reflection mode using Cu radiation, which is usually chosen for fast phase identification. Reflection mode analysis has the advantage that very small samples can be used (though more material is recommended) and the scatter is usually low until high values of 2 theta, so unit cells can be determined with high accuracy. Internal standards are used to control for differences between instruments. XRD sizing limitations. As any method, sizing of crystallites by XRD has limitations. Most importantly, the method has both upper and lower limits, where the lower limit is very much material dependent. Large crystals have narrow reflections, and as rule of thumb, sizes cannot be calculated for crystals larger than 100 nm. As an example, using the first reflection from Anatase as starting point, and using the Scherrer Equation backwards, this gives the expected additional broadening of 0.014. Compared to the contribution from the instrument 0.072 from NRCWE and 0.097 from IMC-BAS, it is seen that the instrument contribution contributes most to the resulting peak. Another issue when calculating the crystal size from X-Ray diffraction is how accurate the results really are. At NRCWE it has been decided to round the sizes to whole numbers and listthose as results; however for the comparison the numbers have been listed with one decimal.The real and important question is however; how accurate are the calculations? It is known that the larger the crystals get, the more the instrument contribution matters. However for very small crystals it is difficult to find the background and thereby the height of the reflection, so in this case it is also difficult to find the right FWHM, and calculate the right size. It was assumed that the results are more uncertain than we have listed. Our estimate is that the uncertainty probably is on the order of ± 5 nm for all the samples.

Details on methods and data evaluation

The SAS are principally amorphous and XRD can therefore not give information on the silica-phase unless it has crystallized or it contains other crystalline (undesired) impurities. Data treatment: Many programs are available for calculation on XRD data can directly calculate the crystal size. It can be quite difficult to find their actual way of calculation, but they are more or less based on the same principles of the Scherrer Equation, stating that the wider the reflections the smaller the crystals. NRCWE have chosen 2 types of software for calculations of the XRD data:

1. The Scherrer equation was used on data from “fityk”, a program only calculating the best fit forthe reflections.
2. TOPAS, reporting both the size based on IB (integral breath) and FWHM (full width at halfmaximum). The crystal size was calculated by the the Scherrer Equation. The width and position of the reflection has been found by using the program “fityk”. No structure isadded in this program, it is merely calculating the best fit of the peak shape. The 0.89 K=shape factor value was used in the eqution. Details of the data treatment, used softwares and data storage can be found in the attached file with the final report.

Data gathering

Instruments

The data from NRCWE were measured at room temperature (25°C) on a Bruker D8 Advanced diffractometer in reflection mode with Bragg-Brentano geometry. The analysis were made using CuKα1 X-rays (1.5406 Å) generated using a sealed Cu X-ray tube run at 40 kV and 40 mA. The x-raybeam was filtered for CuKα2 and focused using a primary beam Ge monochromator and fixeddivergence slit 0.2°. The analyses were made in the stepping mode stepping 0.02 degree 2theta persecond and data were collected using a linear PSD detector (Lynx-eye) with opening angle 3.3°.

Calibration

The analysis were made using CuKα1 X-rays (1.5406 Å) generated using a sealed Cu X-ray tube run at 40 kV and 40 mA. The x-ray beam was filtered for CuKα2 and focused using a primary beam Ge monochromator and fixed divergence slit 0.2°. The analyses were made in the stepping mode stepping 0.02 degree 2theta per second and data were collected using a linear PSD detector (Lynx-eye) with opening angle 3.3°.Each instrument has a unique contribution to the X-ray diffraction profile, which should be documented for detailed data comparisons using e.g., a large crystallite standard. For the analysis,NRCWE used a CeO_2 (NIST SRM674a) standard. To assess the contribution from the instrument,the full width at half maximum, FWHM, was measured on the standard and plotted as a radian angle.

Reproducibility

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

State of test material

other: fluffy powder

Any other information on materials and methods incl. tables

The SAS are principally amorphous and XRD can therefore not give information on the silica-phaseunless it has crystallized or it contains other crystalline (undesired) impurities.The Synthetic Amorphous Silica (SAS) samples were very difficult to mount in a standard sample holder. The sample seem to “jump out” of the sample holders with only the slightest disturbance e.g.when using the glass plate to press the samples into the holders. Instead they were mounted with vacuum grease in a single crystal Si low background sample holder.Measurements of empty sample holder with vacuum grease only showed an amorphous signal in theXRD spectrum.The powder samples were mounted by smearing as little vacuum grease as possible on the Si sample holder. Then the powder sample was topped on the sample holder and vacuum grease. The most important disadvantage of this procedure is a small shift of the zero point, as the sample is not entirely in the correct position.

Results and discussions

Common name

The NM-203 is amorphous.

Overall remarks, attachments

Overall remarks

Estimation of amorphous content based on addition of material is not recommended. It is difficult to ensure an effective mixing and by adding a crystalline material one may shadow the presence ofother materials or the dopant. Results from quantitative determination of bulk phase composition (proportions) and average crystallite sizes may be affected by the settings chosen to mathematically fit the X-ray diffractograms as well as by the type of reference or standard used to obtain the diffractogram. Observations indicating these phenomena have been made in NANOGENOTOX and are currently under investigation.

Attached full study report

Draft_D4.3 NANOGENOTOX Min Chem Composition.pdf / 1.79 MB (application/pdf): ENV/JM/MONO(2015)14/ANN3

Applicant's summary and conclusion

Conclusions

The NM-203 is amorphous however several crystalline impurities were detected impurities detected in NM-203:
Amorphous + Na_2SO_4 : 0 observation
Amorphous + Na_2SO_4 +Boehmite (AlO(OH)) : 3 observations (1 in vial 0152 and 2 in vial 0363)

Cross-reference to other study

http://www.nanogenotox.eu/

4.25 Crystallite and grain size

4.26 Aspect ratio, shape

Endpoint study record: Aspect ratio, shape.001

Administrative Data

Purpose flag	weight of evidence ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type	other: see chapter 4.5 (TEM)
Rationale for reliability	Fumed (pyrogenic) synthetic amorphous silica shows fractal and aggregated stable secondary structures (See chapter 4.5 TEM)

Data gathering

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

4.27 Specific surface area

Endpoint study record: Specific surface area.001

Administrative Data

Purpose flag key study ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Reliability 1 (reliable without restriction)

Materials and methods

Methods

BET

Principles of method if other than guideline

Standard BET (Brunauer, Emmett and Teller) method can be used for calculating the specific surface area from an adsorption isotherm (Brunauer et al, 1938). The BET algorithm requires a type II adsorption isotherm in the range of $0.05 < p/p0 < 0.3$ and a large constant ($c \geq 50$).

Data gathering

Instruments

Equipment: e.g. Gemini or Tristar by manufacturer Micromeritics

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Results and discussions

Specific surface area

Mean 200 m²/g

Standard deviation

Remarks on results including tables and figures

Specified range: 200 ± 25 m²/g

Applicant's summary and conclusion

Conclusions

Surface BET DIN ISO 9277 adsorption of nitrogen at 77.4 K for NM-203 has been determined to: 196 m2/g (Specified range: 200 ± 25 m²/g).

Endpoint study record: Specific surface area by BET by IMC-BAS

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.4: Determination of specific surface area of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	IMC-BAS (BG)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: owner:NANOGENOTOX

Materials and methods

Methods

BET

Principles of method if other than guideline

Surface area and porosity are important characteristics, in understanding the structure, formationa nd potential applications of different natural materials. For this reason it is important to determine and control them accurately. The most widely used technique for estimating surface area is the socalled BET method (Brünauer, Emmett and Teller, 1938) [5]. The concept of the theory is anextension of the Langmuir theory, which is a theory for monolayer molecular adsorption, tomultilayer adsorption with the

following hypotheses: (a) gas molecules physically adsorb on a solid inlayers infinitely; (b) there is no interaction between each adsorption layer; and (c) the Langmuirtheory can be applied to each layer

Details on methods and data evaluation

BET analyzer operates by measuring the quantity of gas adsorbed onto or desorbed from a solid surface at some equilibrium vapor pressure. The data are obtained by admitting or removing a known quantity of adsorbate gas (Nitrogen) into or out of a sample cell containing the solid adsorbent maintained at a constant temperature below the critical temperature of theadsorbate (at temperature of liquid Nitrogen). As adsorption or desorption occurs the pressure in thesample cell changes until equilibrium is established. The quantity of gas adsorbed or desorbed at theequilibrium pressure is the difference between the amount of gas admitted or removed and theamount required to fill the space around the adsorbent (void space).Smaple preparationno dpeciall treatment needed. Measurements performed on powder. 0.1 g of the material placed it in the appropriate cell size (the volume of the sample mayvary from sample to sample due to difference in density etc.). Details of the method and values of used paramters might be found in the attached file with full study report : Draft D4.4_specific surface area

Data gathering

Instruments

High-speed surface area and pore size analyzer NOVA 4200e (Quantachrome)NOVA 4200e equipped with four preparation ports (vacuum or flow degassing) and four analysis ports. It provides single and multi-point BET surface area with y-intercept, ”C” constant, slope and correlation coefficient; up to 100 adsorption and 100 desorption isotherm points; B.J.H pore size distribution calculated from the adsorption or desorption isotherm; total pore volume and average pore radius.

Reproducibility

two measurements were performed for NM-203

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

State of test material

other: fluffy powder

Any other information on materials and methods incl. tables

The results from the BET analyses conducted in the project were compared with manufacturers data.BET (manufacturer) (m^2/g): 200

Results and discussions

Specific surface area

Mean 203.92 m²/g

Standard deviation

Remarks on results including tables and figures
Total pore volume (mL/g): 0.4991Micropore volume (mL/g): 0.0

Overall remarks, attachments

Attached full study report
Draft D4.4_specific surface area.pdf / 1.62 MB (application/pdf): ENV/JM/MONO(2015)14/ANN4

Applicant's summary and conclusion
Conclusions
see the endpoint: compraison between BET and SAXS

Cross-reference to other study
http://www.nanogenotox.eu/

Endpoint study record: Specific surface area by SAXS_CEA

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.4: Determination of specific surface area of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	CEA (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access
other: owner: NANOGENOTOX

Data protection claimed
yes, but willing to share

Materials and methods

Methods
other: SAXS and USAXS

Principles of method if other than guideline
Details of the method can be found in the attached SOP document: SIAR

Details on methods and data evaluation
1) Sample preparation: powder samples were prepared in 1.5 mm glass capillaries leading to typical

equivalent thickness of dense material from 100 to 200 µm. The usual thickness of aqueous samples for SAXS measurement is 1mm with an acquisition time of 1 hour.Dispersions for analysis are typically produced by sonication in a dispersion medium (see each dedicated SOP (general SOP from NANOGENOTOX) for specific dispersion protocols). The concentration required for analysis depends on the relative scattering length densities between particles and dispersion medium, and the density of materials. The sample must be stable within the time-frame of the measurement. Typical concentration in oxide for NANOGENOTOX suspensions is 3 g/L. Since the scattering length density of silica is relatively low, higher concentrations were used when possible.

2) Details on method: Very detailed description of the method could be found in the attached SOP document.In order to calculate the sample transmission, the flux of incident and transmitted beam are measured and averaged over 200 s before running the SAXS measurement. The time of acquisition necessary for SAXS experiment depends on the sample properties. For SiO_2 powders, two measurements were performed: one with a short time of 200 s or 150 s to get unsaturated data for small angles (low q), and one for a long time of 1800 s to get data in the high q region with low signal/noise ratio. For aqueous suspensions prepared for NANOGENOTOX, SAXS measurements were performed in kapton capillaries of internal thickness 1.425 mm and run for 3600s, leading to transmissions of about 0.25. USAXS measurements were performed in 1 mm or 1.5 mm non-sticky double kapton cells
3) Raw Data Treatment-Raw data, translated into intensity as a function of the scattering vector q, are first normalized by parameters of the experiments such as acquisition time, sample thickness and calibration constants determined using reference samples.
   -The data are thus expressed in absolute scale (cm^{-1}).
   -Backgrounds are then subtracted. -SAXS data obtained for short time and long time are concatenated, together with USAXS data to get continuous diffractograms on the whole q range
   -For powder samples, the Porod law is applied to extract specific surface areas of raw materials.
   -Data from suspensions are fitted with a model describing fractal aggregates of primary particles. In this model, the whole q range is divided into sections reflecting different structural levels in the sample, and fitted by local Porod and Guinier scattering regimes.
   -Intensity average parameters are then determined such as radius of gyration for the primaries and for the aggregates, and a fractal dimension for the aggregates.
   -Invariants are calculated, which give a correlation between the sample concentration and the specific surface area obtained in suspension.
4) SSA from SAXS Specific surface area determination from SAXS on powders: To treat raw SAXS data and get absolute intensities, the intensity by the thickness of the scattering material need to be normalised. However, for powder samples, the sample thickness is not well defined and cannot be precisely controlled as it depends on the powder compaction and the different scales of porosity. To elude this problem, a model system is used, considering the effective thickness of material crossed by X-rays, called eB, corresponding to an equivalent thickness if all the material would be arranged in a fully dense (no inner or outer porosity) and uniform layer.Details of the method can be found in the attached file with SOP.

Used Protocols

The attached protocol describes the general procedure applied at CEA/LIONS (Laboratoire Interdisciplinaire sur l'Organisation Nanométrique et Supramoléculaire) to perform Small Angle X-ray Scattering measurements and to treat the data to extract physic-chemical properties of materials. This procedure was applied in the framework of NANOGENOTOX among others to characterize SiO_2 manufactured nanomaterials as raw powders and SiO_2 in aqueous suspensions.

Used Protocols: attached files

Attached document SOP_SAXS_CEA.doc / 2.38 MB (application/msword): SIAR

Remarks Protocol for SAXS measurments in CEA laboratories

Data gathering

Instruments

The main set up components used for SAXS and USAXS experiments at CEA/LIONS:
♣ X-ray generator : Rigaku generator RUH3000 with copper rotating anode (λ= 1.54 Å), 3kW♣ Home made optic pathways and sample holders (with two channel-cut Ge (111) crystals in Bonse/Hart geometry for USAXS set up, cf Lambard (1992).
♣ Flux measurement for SAXS set up : pico amperemeter Keithley 615
♣ Flux measurement for USAXS set up : DonPhysik ionization chamber
♣ Detector for SAXS set up : 2D image plate detector MAR300
♣ Detector for USAXS set up: 1D high count rate CyberStar X200 associated to a scintillator/ photomultiplier detector.All experimental parameters are monitored by computer by a centralized control-command system based on TANGO, and interfaced by Python programming. 2D images are treated using the software ImageJ supplemented with some specific plugging developed at CEA/LIONS. This control-command system has been achieved by Olivier Taché and is detailed in: O. Taché ; « Une architecture pour un système évolutif de contrôle commande d'expériences de physique », Engineer thesis, 2006, available at http://iramis.cea.fr/sis2m/lions/tango/tango-ds/memoire.pdf

Calibration

- A sample of 3 mm of Lupolen® (semi crystalline polymer) was used for the calibration of the intensity in absolute scale, the maximum intensity being adjusted to 6 cm^{-1}.
- A sample of 1 mm of octadecanol was used for the calibration of the q range (calculation of sample-to-detector distance), the position of the first peak standing at 0.1525 $Å^{-1}$.
- Calibrations in intensity and in q range were performed before each series of measurements.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

State of test material

other: fluffy powder

Results and discussions

Specific surface area

Mean 167 m^2/g

Standard deviation 13 m^2/g

Remarks on results including tables and figures

see report

Overall remarks, attachments

Attached full study report

Draft D4.4_specific surface area.pdf / 1.62 MB (application/pdf): ENV/JM/MONO(2015)14/ANN4

Applicant's summary and conclusion

Conclusions

see the endpoint: comparison between SAXS and BET

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Specific surface area comparison between SAXS and BET results

Administrative Data

Purpose flag key study ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Data access

other: owner: NANOGENOTOX

Materials and methods

Principles of method if other than guideline

see the conclusions

Applicant's summary and conclusion

Conclusions

Comparision between SAXS and BET results.T he results from both analytical methods show a difference for NM203 material. BET specific surface area (m^2/g): 203.92SAXS specific surface area (m^2/g): 167.2. Assessed from the methodology, most of the differences may be explained by the combined errors in density and placement of plateau. Other explanations may come from the difference in thermal treatment and outgassing of the powders before BET analysis.

Executive summary

The samples were analyzed for their specific surface area using BET and SAXS, which are two different analytical methods relying on nitrogen gas adsoption and X-ray scattering, respectively. Proof of principle has been shown for SAXS analysis of all three compounds (TiO_2 amorphous silica and CNT) for the deduction of surface area is applicable. However, there is not an overall linear correlation between SAXS and BET data. The SAXS appears to underscore the specific surface area determined by BET. In this assessment, one must also consider the differences and limits of the methods. The determination of surface area for very small and bigger (>200 nm) particles needs more attention.The BET results given by producers are generally in very good agreement with the NANOGENOTOX data. This suggests that producer instrumental capacity and the SOPs for making BET analysis are similar or of same quality as the procedures used in NANOGENOTOX. All being well as SAXS data confirms the obtained results.

4.28 Zeta potential

Endpoint study record: Zeta potential.001

Administrative Data

Purpose flag	weight of evidence ( ) robust study summary ( ) used for classification ( ) used for MSDS
Data waiving	other justification
Justification for data waiving	Currently, no standard method is available without definition of boundary conditions, like dispersion medium, concentration, pH value, conductivity, ion background. Therefore, a substantiated conclusion is not possible and it could not be determined.

Data gathering

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	7631-86-9
Identifier	EC number
Identity	231-545-4
Identifier	IUPAC name
Identity	dioxosilane

Endpoint study record: Zeta potential_by _Zeta-metry_CEA

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	Keld Alstrup Jensen	**Year**	
Title	Deliverable 4.5: Nanomaterial datasets with requested physicochemical properties. Surface charge, hydrodynamic size and size distributions of NM in aqueous suspensions by zetametry, dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS)		
Bibliographic source			
Testing laboratory	CEA (F)	**Report no.**	
Owner company	NANOGENOTOX		
Company study no.		**Report date**	

Data access

other: data owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Methods

Laser-Doppler-Electrophoresis

Details on methods and data evaluation

- Electrophoretic mobility is measured by a combination of laser Doppler velocimetry, a technique based on the phase shift of the laser beam induced by the movement of particles under an electric field, and phase analysis light scattering (patented M3-PALS technique).
- In this “mixed mode measurement” (M3), the measurement consists of the application of an alternative electric field in two modes, a fast field reversal mode, and a slow field reversal mode.
- The light scattered at an angle 17° is combined with the reference beam and the resulting signal is treated by the computer.
- During the fast field reversal mode, the electro-osmose effect is negligible, allowing to determine an accurate mean zeta potential, whereas the slow field reversal mode helps modelling the distribution of potentials.
- More details on the results of zeta potential measurements with the M3-PALS technique are available in the documentation from Malvern Instruments and can be downloaded from http://www.malvern.com, application library section.

Used Protocols

The SOP is developed by CEA and it is different from the Nanogenotox SOP. The details of the procedure can be found in the attached files with SOP

1) Sample preparation:Samples for zeta potential measurements are prepared as aqueous suspensions of 1 g/L for SiO_2 nanomaterials with constant ionic strength of 0.036 mol/L (monovalent salt) and controlled pH. They are prepared by dilution of concentrated sonicated stock suspensions of 10 g/L into pH and ionic strength controlled "buffers" prepared by addition of HNO_3, NaOH and $NaNO_3$ in various proportions.

20 mL of stock suspensions of 10 g/L NM in pure water are prepared as follows:

- 200 mg of NM are weighed and introduced in a 20 mL gauged vial (with protective gloves, mask and glasses, and damp paper towel around the weigh-scale).
- The 20 mL gauged vial is completed with ultrapure water (MilliQ)
- The suspension is transferred into a flask suitable for sonication (a 40 mL large-neck glass flask of internal diameter 38 mm was used, height of 20 mL liquid 20 mm), making sure that all the settling material is recovered.
- The suspension is dispersed by ultrasonication for 20 min at 40% amplitude in an ice-water bath. Probe, sample and bath are placed in a sound abating enclosure, and inside a fume hood.

2) Preparation of "buffer" solutiono Denominated "buffer" solutions are aqueous ionic solutions of Na^+, H^+, NO_3^- and OH^-, designed to display the same ionic strength with a modulated pH.
   - A first set of concentrated buffer solutions (0.1 mol/L of salt, various pH) are prepared by addition of HNO_3, NaOH and $NaNO_3$ in various proportions in ultrapure water.
   - Then 20 mL of these concentrated buffers are poured into 50 mL gauged vials completed with ultrapure water, giving a new set of buffers with a salt concentration of 0.04 mol/L and a pH ranging from 1.5 to 12.5. The combination of the two buffers gives access to the necessary intermediate pH.
   - By this procedure, acidic buffers contain 0.04 mol/L of NO_3^- and various ratios of Na^+/ H^+ as counter ions; likewise, basic buffers contain 0.04 mol/L of Na^+ and various ratios of $NO3^-/OH^-$.
3) Preparations of suspensions for zeta potential measurements and determination of isoelectric pointIn this SOP

Zeta potential measurements are performed on 1 g/L suspensions for SiO_2 samples.

- 10 g/L suspensions of the SiO2 samples are used right after sonication.
- Series of samples are prepared by addition of 400 µL of concentrated NM suspension and 3.6 mL of 0.04 mol/L buffer solutions in a 5 mL glass flask.
- This leads to samples of 1 g/L SiO_2 and a constant ionic concentration of 0.036 mol/L in monovalent salt.
- For each NM, an additional sample is prepared in MilliQ or Nanopure water with the same NM concentrations, i.e. by addition of 400 µL of concentrated NM suspension and 3.6 mL of water.

Used Protocols: attached files

Attached document	SOP for surf charge isoele p by zetametry CEA.doc / 391.5 KB (application/msword): SIAR
Remarks	The SOP is developed by CEA and it is different from the Nanogenotox SOP.
Attached document	SOP for dispersion of Sio2 by CEA.doc / 330 KB (application/msword)
Remarks	The SOP is developed by CEA and is different than NANOGENOTOX SOP.

Data gathering

Instruments

♣ Zetasizer Nano ZS (e.g, Malvern Instruments), equipped with laser 633 nm

♣ Autotitrator (Malvern MPT-2) –optional for automatic determination of IEP
♣ Malvern software (DTS 5.03 or higher) installed on a computer to control the Zetasizer
♣ Clear, disposable zeta cells (DTS1061 - DTS1060C)

Calibration

- Equilibrium pH of the suspensions are measured and considered as pH values for the reported results.
- The suspension to be characterized by zetametry are inserted in Malvern patented folded capillary cells with gold electrodes (volume 0.75 to 1 mL), DTS1061.
- Zeta measurements (electrophoretic mobility) are performed on the "general purpose" mode at 25C with automatic optimization of laser power, voltage settings, the number of runs (10 - 100) and run duration, and repeated 3 times with no equilibration time as the sample is already at ambient temperature.
- The Smoluchowski model (F(κa)=1.5) was used, considering the high polarity of aqueous solvent, and hence a thin double layer around the particles. o For the dispersant, the refractive index Ri, absorption Rabs, viscosity and di-electric properties considered are the ones of pure water.
- The parameters used for dispersant and material properties are available in the attached file with the SOP for Zetametry.
- For each suspension of known pH, fixed ionic strength and fixed NM concentration, the measurements for determining the zeta potential are performed on a general purpose mode with automatic determination of measurement parameters.
- Three measurements are performed and averaged for reporting.
- For unstable samples, measurements are performed on supernatants.
- Zeta potentials are then plotted against pH to determine the stability domains and isoelectric points (IEP)-see attached file with the figure.

Reproducibility

- Three measurements are performed and averaged for reporting. o For unstable samples, measurements are performed on supernatants.
- The reported value is the average of zeta potential values from the 3 measurements (determined during the fast field reversal step), with possible exclusion of diverging data.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

State of test material

dispersion

Results and discussions

Zeta potential

Mean see the attachment with the figure

Standard deviation

In medium (specify)

suspensions (1 g/L) in constant ionic strength aqueous media (0.036 mol/L HNO_3/NaOH)

Remarks on results including tables and figures

NM-203 forms a stable suspension, with negatively to neutral charged particles. The zeta potential varied greatly as function of pH and reached -35 mV around pH 7. IEP 2-4

Overall remarks, attachments

Attached background material

Attached document zeta potential as a function of pH CEA SiO2.doc / 179.5 KB (application/msword): ENV/JM/MONO(2015)14/ANN5

Remarks

Attached full study report

Draft D4.5 ZETA DLS SAXS analysis.pdf / 2.03 MB (application/pdf)

Applicant's summary and conclusion

Executive summary

For each suspension of known pH, fixed ionic strength and fixed NM concentration, the measurements for determining the zeta potential are performed on a general purpose mode with automatic determination of measurement parameters. Three measurements are performed and averaged for reporting. For unstable samples, measurements are performed on supernatants. Zeta potentials are then plotted against pH to determine the stability domains and isoelectric points (IEP). NM-203 forms a stable suspension, with negatively to neutral charged particles. The zeta potential varied greatly as function of pH and reached -35 mV around pH 7. IEP 2-4

Cross-reference to other study

http://www.nanogenotox.eu/

4.29 Surface chemistry

Endpoint study record: Surface chemistry.001

Administrative Data

Purpose flag weight of evidence ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type other: See chapter 1.4 IR Spectroscopy

Rationale for reliability Fumed (pyrogenic) synthetic amorphous silica provided to the sponsoring program is hydrophilic silicon dioxide. It has a higher surface energy (solid) than the surface tension of water, which is 72 mN/m (same dimension as mJ/m² solid). Therefore, water will wet hydrophilic silicon dioxide. The high surface energy is linked to the hydrophilic silanol groups at the surface. Different silanol structures have been characterized, isolated, vicinal and geminal silanols (See IR Spectroscopy).

Data gathering

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

4.30 Dustiness

Endpoint study record: Dustiness by Small Rotating Drum (SD) method by NRCWE

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.6: Dustiness of NANOGENOTOX nanomaterials using the NRCWE small rotating drum and the INRS Vortex shaker		
Bibliographic source			
Testing laboratory	NRCWE (DK)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Methods

other: Small Rotating Drum method

Principles of method if other than guideline

The small rotating drum was designed as a downscaled version of the EN 15051 rotating drum whilemaintaining important test parameters.

Details on methods and data evaluation

- The small rotating drum was designed as a downscaled version of the EN 15051 rotating drum whileo maintaining important test parameters. This enabled testing of smaller material amounts (~6g instead of ~500g).
- The drum consists of a cylindrical part [internal diameter (i.d.) 16.3 cm, length 23.0 cm, volume 4.80 l] with a truncated cone at each end (half angle 45°, length 6.3 cm, volume 1.13 l). The total volume of the drum is 5.93 l.
- The drum was made of stainless steel and all inside surfaces were polished to 450 ± 50 gloss units to minimize surface adhesion and to facilitate cleaning. o The drum was electrically grounded as prescribed by EN 15051.
- The drum contains three lifter vanes (2 x 22.5 cm). In EN 15051, a 1-min rotation at 4 rpm and eight lifter vanes are prescribed. Therefore, the present drum was operated at 11 rpm to obtain the same number of powder parcels falling per minute as in the EN 15051 test (Schneider and Jensen, 2008).
- The inlet air to the drum was controlled at 50 % RH and HEPA-filtered to ensure no particle background.o In the applied set-up, respirable dust is collected by a GK2.69 respirable dust sampler at 4.2 lpm (BGI, UK) and dust particle size-distributions are measured using the Fast Mobility Particle Sizer (FMPS 3091, TSI), with a range of 5.6 to 560 nm, and the Aerodynamic Particle Sizer (APS 3321, TSI) with a range of 0.5 to 20 μm. It is important to note that these two instruments provide a size distribution which is expressed for the FMPS in electric mobility equivalent diameter, whereas for the APS, it is the equivalent aerodynamic diameter that is

obtained. A GRIMM CPC may be connected for simultaneous number-concentration measurements, but not used in this study.

- The dustiness test was conducted in triplicates for each powder preceded by a so-called saturation run completed to coat all inner surfaces of the system with dust.
- The saturation test was performed using 2 grams of powder and rotation for 60 seconds.
- Then the actual triplicate tests were completed using 6 grams of test material per run.
- After each run the drum was emptied by pouring out the residual powder and gently tapping the drum three times with a rubber hammer.
- When loading the powder in the drum, it was carefully placed centrally in the drum on the upwards moving side of of of three inner lifter vanes placed at bottom position.
- Then the drum was sealed followed by 60 seconds of background measurements were done to ensure a particle free test atmosphere. o The experiment was then initiated by rotating the drum for 60 seconds during which particles were emitted and led through the airflow to the sampling train. o After the drum was stopped, measurements and sampling was continued for additional 120 sec to catch the remaining airborne particles in the dust cloud. Thus, the total time during which the measurement is made is 180 s.
- This then completed the rotational test. The drum and sampling lines were thoroughly cleaned between each powder type using a HEPA-filter vacuum cleaner designed for asbestos cleaning and wet-wiping. Then the drum was let to air-dry before the next powder could be tested.
- The mass of collected respirable dust was determined after conditioning the filters and controls in our weighing room (22°C; 50 %RH) using a Sartorius microbalance (Type R162 P; Sartorius GmbH, Göttingen, Germany). The mass is used to categorize the dustiness levels of the powders according to EN15051.
- Additional information may be found in the attached detailed final report on dustiness measurements.

Data gathering

Instruments

In the applied set-up, respirable dust is collected by a GK2.69 respirable dust sampler at 4.2 lpm (BGI, UK) and dust particle size-distributions are measured using the Fast Mobility Particle Sizer (FMPS 3091, TSI), with a range of 5.6 to 560 nm, and the Aerodynamic Particle Sizer (APS 3321, TSI) with a range of 0.5 to 20 µm. It is important to note that these two instruments provide a size distribution which is expressed for the FMPS in electric mobility equivalent diameter, whereas for the APS, it is the equivalent aerodynamic diameter that is obtained. A GRIMM CPC may be connected for simultaneous number-concentration measurements, but not used in this study.

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

State of test material

other: fluffy powder

Results and discussions

Remarks on results including tables and figures

Test mass (g): 1.5
Dustiness index Number (1/mg) CPC: 6.30E+06
Inhalable (Mass (mg/kg)): 5800 (± 1488)Respirable (Mass (mg/kg)): 218 (± 24)

Overall remarks, attachments

Overall remarks

The powder generate fine aerosol with an electrical mobility equivalentpeak diameter typically between 200 and 300 nm. Larger μm-size-modes are present in all samples, but none of the coarse mode particle concentrations exceed the 200-300 nm mode-size particle concentrations.

Attached full study report

Draft Deliverable D4-6_Dustiness.pdf / 1.23 MB (application/pdf): ENV/JM/MONO(2015)14/ANN6

Applicant's summary and conclusion

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Dustiness by Vortex Shaker (VS) method by INRS

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.6: Dustiness of NANOGENOTOX nanomaterials using the NRCWE small rotating drum and the INRS Vortex shaker		
Bibliographic source			
Testing laboratory	INRS (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Details on methods and data evaluation

Vortex Shaker (VS) methodThe vortex shaker method (VS) consists of a centrifuge stainless tube agitated by a vortex in which the testpowdered material is placed together with 100 μm diameter bronze beads. These are used to help the deagglomeration of powders.HEPA filtered air, controlled at 50% RH, pass through the tube in order to transfer the released aerosol to the sampling and measurement section. The protocol developed for the experiments performed within this project used two different versions of the sampling and measurement section. All tests were conducted with VS method using approximately 0.5 ml powder, which is placed in the sample vialtogether with 5 g bronze beads (100 μm), used to agitate and de-agglomerate the powder. The sample is allowed conditioning in the 50% RH before the shaker for a powder agitation period of 3600 s (60 min). Two different setup version were developed. The first version is devoted for real-time measurement using ELPITM Classic (10 Lpm, Dekati) for size distributions according to the equivalent aerodynamic diameter and CPC (Model 3786 UWCPC, TSI) for number concentrations. This version is also devoted for collecting airborne particles for subsequent electron microscopy (EM) observations. The test on the sample has been performed three times with this setup.
The results of the tests performed with this first version of the VS method leads to the determination of:
- Dustiness indices expressed as the total number of particles emitted (based on data from CPC).
- Particle size-distribution of the aerosol (based on data from ELPITM Classic in its standard configuration).The CPC used was the Model 3785 Water-based Condensation Particle Counter (TSI, USA).
This CPC detects particles from 5 to >3000 nm. It provides a wide, dynamic, particle-concentration range, an essential characteristic for the tests considered. Featuring a single-particle-counting mode with continuous, live-time coincidence correction and a photometric mode, the CPC measures particle number concentrations up to 107 particles/cm^3 with high accuracy.ELPI™ (Electrical Low Pressure Impactor) is an instrument to measure airborne particle size distribution and concentration in real-time. It operates in the size range of 7 nm – 10 μm in its standard configuration. Because of its wide particle size range and rapid response (< 5 s), the ELPI™ has been considered an ideal measurement instrument for the analysis of the unstable concentrations and size distributions, or the evolution of size distributions that could be observed in these tests. In order to prevent particle bounce and charge transfer during the tests, all collection substrates used (PVC GELMAN GLA-5000 5μm / 25 mm) have been greased.In the ELPI the measured current signals are converted to (aerodynamic) size distribution using particle size dependent relations describing the properties of the charger, the impactor stages, and the effective density of the particles. The particle effective density provides a relationship between mobility and aerodynamics sizes. Effective density is a parameter which is complex to measure (Olferta et al., 2007), and values for samples used in the project are not available in the literature. Therefore, the following assumption has been made for the data from the ELPI: spherical particle with a density equal to the density of the condensed phase of the material constituting the NM. Density used for NM 200 was: 2.2 g/cm^3 based on Kim et al.(2009). If this assumption is questionable, there is no robust method that can be applied to polydispersed aerosols over a wide size range, such as those used in the project. However, to assess the effect of this parameter on the results, the number size distributions were also calculated for a density of 1 g/cm^3. The details of the calculation can be found in the attached file with the full report. To get information on particle morphology of the emitted aerosol, a simple but specific sampling set-up has been designed (see attached file with the full report). Transmission electron microscope (TEM) copper grids were taped onto 25 mm diameter polycarbonate membrane filters (0.4 or 0.8μm). Fiber backing filters were used to support the polycarbonate filters. Air flow was driven by a personal sampling pump at a flow rate of 1 L/min. The duration of the sampling has been set to 1 hour. The sampling period was set equal to the duration of a test (1 hour). For some test, the sample was accumulated over two trials in order to have enough particles to observe. Different TEM copper grids having different carbon have been used (Carbon film, Quantifoil Holey Carbon Films or Holey Carbon Support Film). It is important to note that the duration of the test is to be considered as the process is dynamic. In the original INRS protocol

developed, the duration of a test was set equal to 3600 s. But in the first version of the set-up as the instruments measure in real time, it is possible to perform the calculation for different durations between 0 and 3600 s. In this report, the calculations based on the CNC data were performed for two durations: 180 s and 3600 s. The first duration (180 s) was chosen to be consistent with the method SD. For the second version of the setup, the duration of the test was set to 3600 s, which corresponds to the originalprotocol of the Vs method.
The second version of the setup is used for collecting respirable mass fraction of the emitted aerosol. The respirable mass fraction is obtained by sampling with a GK2.69 cyclone (BGI, UK). The filters have been preweighed and post-weighed following the recommendations of the ISO 15767:2009 on the same analytical balance. Only one test was performed with this setup due to time constraints. This is why the results are not presented with a confidence interval based on reproducibility. However, measurement uncertainty has been calculated for each measurement performed. The dustiness index in respirable mass (mg) of particles per kilogram, was calculated as the respirable mass of generated particles in milligrams divided by the total mass of the test NM sample in kilograms: The recommendations of the standard ISO 15767:2009 were followed to determine the LOD of the weighing procedure for the filters used for sampling respirable mass of particles during this project. The LOD for the PVCGELMAN GLA-5000 (5 µm/37 mm) filters was equal to 20 ng. This value is used to determine the LOD expressedin dustiness index.The flow diagram of the experimental protocol used for the NGT project can be found in the attached file with full study report. The preparation of NM samples for VS testing include:

1) to take a series of 7 samples of 0.5 cm^3 of the vial containing the nanomaterial received at the laboratory in this project,
2) to accurately weigh the samples.Three of the samples are devoted for test with the first version of the set-up (real-time measurement), one for the second version (respirable mass fraction measurement,) and three for the gravimetric water content measurement.

Any additional samples are intended to further testing that would be needed in case of default validation. Microcentrifuge graduated tubes with secure seals and caps have been chosen to keep the 0.5 cm^3 samples.The gravimetric water content was performed using a HR83 Halogen Moisture Analyzer (Mettler Toledo) and following a drying program defined specifically for small quantities of used NM (Temperature = 160°C; duration = 170 s).
The weighing of the NM samples was performed with a XP205 analytical balance (10 µg readability, Mettler Toledo) while the weighing of the 37-mm filters from the respirable sampler was performed with a MX5 microbalance (1 µg readability, Mettler Toledo). Particular attention was given to the experimental device cleaning between successive tests. All pipes and other connections were systematically cleaned with water and/or ethanol and dried in an oven, or eventually changed. The checking of the airflows was performed using a primary flow bubble calibrator (Gillian® Gillibrator 2). Prior to each test, the cleanliness of the air was assessed on the basis of measurements made using the CNC. In the case of a non-compliant result, everything was taken from the beginning.The validation of a test depends on several factors such as:

1) the stability of the parameters during the test,
2) a good reproducibility of measured number concentrations,
3) a good sequence of steps for the respirable aerosol sampling etc. The entire set-up was located inside a variable volume fume hood to prevent exposure of the operator. Similarly, all operations like weighing, water content measurement and sample preparation were carried out in a specific containment system that has a unique turbulent-free, low flow design which allows sensitive balance to operate without fluctuation and protects the operator from exposure to airborne particles that could be released when handling and weighing NM samples.

Used Protocols

The recommendations of the standard ISO 15767:2009 were followed to determine the LOD of the weighing procedure for the filters used for sampling respirable mass of particles during this project.

Data gathering

Instruments

1st setup: ELPITM Classic (10 Lpm, Dekati) for size distributions CPC (Model 3786 UWCPC, TSI) for number concentration ssubstrates used PVC GELMAN GLA-5000 5µm / 25 mm.
Different TEM copper grids having different carbon have been used (Carbon film, Quantifoil Holey Carbon Films or Holey Carbon Support Film). TEM not specified
Second setup: The respirable mass fraction is obtained by sampling with a GK2.69 cyclone (BGI, UK). The gravimetric water content was performed using a HR83 Halogen Moisture Analyzer (Mettler Toledo) The checking of the airflows was performed using a primary flow bubble calibrator (Gillian® Gillibrator 2)

Calibration

Particular attention was given to the experimental device cleaning between successive tests. All pipes and other connections were systematically cleaned with water and/or ethanol and dried in an oven, or eventually changed. The checking of the airflows was performed using a primary flow bubble calibrator (Gillian® Gillibrator 2). Prior to each test, the cleanliness of the air was assessed on the basis of measurements made using the CNC. In the case of a non-compliant result, everything was taken from the beginning.The validation of a test depends on several factors such as:

1) the stability of the parameters during the test,
2) a good reproducibility of measured number concentrations,
3) a good sequence of steps for the respirable aerosol sampling etc. The entire set-up was located inside a variable volume fume hood to prevent exposure of the operator. Similarly, all operations like weighing, water content measurement and sample preparation were carried out in a specific containment system that has a unique turbulent-free, low flow design which allows sensitive balance to operate without fluctuation and protects the operator from exposure to airborne particles that could be released when handling and weighing NM samples.

Compliance with standard (ISO/CEN/other)

yes The recommendations of the standard ISO 15767:2009 were followed to determine the LOD of the weighing procedure for the filters used for sampling respirable mass of particles during this project

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

Results and discussions

Dustiness Index

Mean mg/kg

Standard deviation

Remarks on results including tables and figures

Gravimetric water content and bulk density Sample mass (mg) 119
Water content (wt % dry) 1%Bulk density (g/cm3) 0.03Dustiness indexTime = 180s
Number (1/g) CPC (S.D): 4.1E+05 (1.1E+04)

ELPIa (S.D): 1.6E+06 (2.0E+05)
Time=3600s CPC (S.D): 2.1E+07 (1.1E+07)
Respirable mg/kg (S.D): 51000 (4.55E-02)S.D=standard deviation calculated over 3 repeats

Overall remarks, attachments

Attached full study report

Draft Deliverable D4-6_Dustiness.pdf / 1.23 MB (application/pdf): ENV/JM/MONO(2015)14/ANN6

Applicant's summary and conclusion

Conclusions

Within this project two methods for characterizing the dustiness of nanomaterials in powder have been developed Small Rotatting Drum method and Vortex Shaker Method. The results of the present work suggest that:
- There are different dust generation rate time profiles. This difference in the dynamic of dust generation is reflected in the difference dustiness indices that are calculated.
- Both SD and VS methods gave reproducible results in terms of amount and size distribution of the generated particles for the NM samples in the project.
- All size distributions of as measured by the SD method were bi- or multimodal.
- Airborne particles generated during these tests are agglomerates/aggregates as shown by the few EM observations made on three selected NM. These results are in agreement with those of the existing literature.
- The comparison between the small drum and Vortex shaker shows that no significant correlation between thetwo can be found. Further evaluation of this method is needed in order to link it the standardized rotating drum method. Dustiness as quantified by particle number or by mass-based dustiness index had for both methods a large range. These findings suggest a corresponding large difference in exposure potential. It is however difficult tos ay more to the extent the relationship between index Dustiness and actual exposure is not known. The comparison between the small drum and Vortex shaker shows that no significant correlation between the two can be found. Further evaluation of this method is needed in order to link it the standardized rotating drum method. Dustiness is not an intrinsic physical or chemical defined property of a powder, but its level depends on as well as characteristic properties of the powders and the activation energy in the simulated handling. Therefore different values may be obtained by different test methods (test apparatus, operation procedure, sampling and measurement strategy, etc.). It seems obvious that the absence of a harmonized approach concerning the measurement strategies and techniques, metrics and size ranges and the procedures of data analysis and reporting severely limits the comparison of these dustiness methods. Very little work has been done so far in this direction. That is why such a harmonized approach has been already integrated into various European research programs to be launched soon. One of them will be realized within the framework of the Mandate 461. Dustiness data obtained within this project can therefore contribute with information on the potential exposure risk level during powder handling (Schneider and Jensen, 2009). Size-distribution analysis of dustiness materials additionally may give information on the potential aggregate and agglomerate size of dust particlesreleased from handling.

Cross-reference to other study

http://www.nanogenotox.eu/

4.31 Porosity

Endpoint study record: Porosity.001

Administrative Data

Purpose flag key study ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Materials and methods

Methods

BET Method applied: nitrogen adsorption at 77.4 K

Principles of method if other than guideline

IUPAC distinguishes between micropores d < 2 nm, mesopores 2 < d < 50 nm and macropores d > 50 nm.

Data gathering

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Overall remarks, attachments

Overall remarks

NM-203 is non-porous as regards its primary particles, but the interstitial (void) volume within the agglomerates falls into the macropore range.

Applicant's summary and conclusion

Conclusions

NM-203 is non-porous as regards its primary particles, but the interstitial (void) volume within the agglomerates falls into the macropore range.

Endpoint study record: Porosity by BET by IMC-BAS

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Data source

Reference

Reference type	study report		
Author	KA Jensen	**Year**	2013
Title	Deliverable 4.4: Determination of specific surface area of NANOGENOTOX nanomaterials		
Bibliographic source			
Testing laboratory	NRCWE (DK)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Methods

BET

Principles of method if other than guideline

Surface area and porosity are important characteristics, in understanding the structure, formation and potential applications of different natural materials. For this reason it is important to determine and control them accurately. The most widely used technique for estimating surface area is the so called BET method (Brünauer, Emmett and Teller, 1938) [5]. The concept of the theory is an extension of the Langmuir theory, which is a theory for monolayer molecular adsorption, to multilayer adsorption with the following hypotheses:

a. gas molecules physically adsorb on a solid in layers infinitely;
b. there is no interaction between each adsorption layer; and
c. the Langmuir theory can be applied to each layer.

Details on methods and data evaluation

BET analyzer operates by measuring the quantity of gas adsorbed onto or desorbed from a solid surface at some equilibrium vapor pressure. The data are obtained by admitting or removing a known quantity of adsorbate gas (Nitrogen) into or out of a sample cell containing the solid adsorbent maintained at a constant temperature below the critical temperature of the adsorbate (at temperature of liquid Nitrogen). As adsorption or desorption occurs the pressure in the sample cell changes until equilibrium is established. The quantity of gas adsorbed or desorbed at the equilibrium pressure is the difference between the amount of gas admitted or removed and the amount required to fill the space around the adsorbent (void space).

Data gathering

Instruments

High-speed surface area and pore size analyzer NOVA 4200e (Quantachrome)NOVA 4200e equipped with four preparation ports (vacuum or flow degassing) and four analysis ports. It provides single and multi-point BET surface area with y-intercept, ”C” constant, slope and correlation coefficient; up to 100 adsorption and 100 desorption isotherm points; B.J.H pore size distribution calculated from the adsorption or desorption isotherm; total pore volume and average pore radius.

Reproducibility

double test

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM-203

Test material identity

Identifier CAS number

Identity 7631-86-9

State of test material

other: fluffy powder

Results and discussions

Remarks on results including tables and figures

total pore volume (ml/g): 0.4991micropore volume (ml/g): 0.0

Overall remarks, attachments

Attached full study report

D4 4_specific_surface_area.pdf / 3.56 MB (application/octet-stream): ENV/JM/MONO(2015)14/ANN4

Applicant's summary and conclusion

Cross-reference to other study

http://www.nanogenotox.eu/

4.32 Pour density

4.33 Photocatalytic activity

4.34 Radical formation potential

Endpoint study record: Radical formation potential.001

Administrative Data

Purpose flag	( ) robust study summary ( ) used for classification ( ) used for MSDS
Data waiving	other justification
Justification for data waiving	NoneNote: This specific endpoint characteristic is inherent to the substance and is not linked to a specific lot.

Data gathering

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier	CAS number
Identity	7631-86-9
Identifier	EC number
Identity	231-545-4
Identifier	IUPAC name
Identity	dioxosilane

5. ENVIRONMENTAL FATE AND PATHWAYS

5.1 Stability

5.1.1 Phototransformation in air

Endpoint summary: Phototransformation in air

Administrative Data

Short description of key information

Not applicable. Derived from UV-VIS spectra. EPA OPPTS 835.2310. The spectrum does not show any absorption in the sensitive wavelength range between 270 and 800 nm. Note: This specific endpoint characteristic is inherent to the substance and is not linked to a specific lot ù.

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

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 19 Nov. 1991 - 23 Nov. 1991

Reliability 1 (reliable without restriction)

Rationale for reliability GLP guideline study

Data source

Reference

Reference type	study report		
Author	Hooftman RN, van Drongelen-Sevenhuijsen D	**Year**	1992
Title	The acute toxicity to Brachydanio rerio (OECD guideline 203, 96 h)		
Bibliographic source	Unpublished		
Testing laboratory	TNO Institute of Environmental Sciences, Delft/NL	**Report no.**	IMW-91-0050-02
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 92-0140-DGO	**Report date**	1992-09-23

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Test guideline

Qualifier according to

Guideline OECD Guideline 203 (Fish, Acute Toxicity Test)

Deviations

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS name

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

approx. 99.8 % (SiO_2): CAS-Name: Silica, amorphous,fumed, cryst.-free; CAS-No.: 112945-52-5

Analytical monitoring

no

Vehicle

no

Details on test solutions

PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances)
- Method: stirring for 20 h, then allowed to stand for 4 h before testing;
- Eluate: no, suspensions were tested- Differential loading: yes, 1000 + 10,000 mg/L
- Controls: Synthetic medium from groundwater
- Evidence of undissolved material (e.g. precipitate, surface film, etc): The resulting suspensions at the beginning of the test were homogeneous and milky, at the end of the test in addition a layer of white, starchy flocks on the bottom of the vessels was observed.

Test organisms

Test organisms (species)

Brachydanio rerio (new name: Danio rerio)

Details on test organisms

TEST ORGANISM
- Common name: Zebra fish- Strain:
- Source: M.B. Ruysbroek B.V./Noordvliet 159, Maassluis
- Age at study initiation (mean and range, SD):
- Length at study initiation (length definition, mean, range and SD): 2.2 ± 0.2 cm
- Weight at study initiation (mean and range, SD): 0.09 ± 0.02 g
- Method of breeding:
- Feeding during test: no

ACCLIMATION
- Acclimation period: no data
- Type and amount of food: no data
- Feeding frequency: no data

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

96 h **Remarks**

Post exposure observation period

none

Test conditions

Hardness

204 mg/L as CaCO3

Test temperature

24.5 - 25.3 °C

pH

7.3 - 8.2

Dissolved oxygen

7.1 - 8.2 mg/L

Salinity

not applicable

Nominal and measured concentrations

1000 and 10,000 mg SiO_2/L (nominal)

Details on test conditions

TEST SYSTEM
- Test vessel:
- Type (delete if not applicable): open
- Material, size, headspace, fill volume: glass beaker, 2 L, 1000 ml test suspension
- Aeration: slight aeration (no data)
- Type of flow-through (e.g. peristaltic or proportional diluter): --
- Renewal rate of test solution (frequency/flow rate): no
- No. of organisms per vessel: 10
- No. of vessels per concentration (replicates): 2
- No. of vessels per control (replicates): 2

- No. of vessels per vehicle control (replicates): --
- Biomass loading rate: approx. 0.9 g fish/L

TEST MEDIUM / WATER PARAMETERS
- Source/preparation of dilution water: prepared from groundwater by addition of several salts
- Total organic carbon: 1.7 mg/L
- Alkalinity: -- no data
- Ca/Mg ratio: 1.83
- Conductivity: --

OTHER TEST CONDITIONS
- Adjustment of pH: no
- Photoperiod: 16 h light, 8 h dark
- Light intensity: -- no data

EFFECT PARAMETERS MEASURED (with observation intervals if applicable):
survival. swimming behaviour, colour, respiratory function, morphological and physiological changes that were visually observable at 24, 48, 72, and 96 h

TEST CONCENTRATIONS
- Spacing factor for test concentrations: 10
- Justification for using less concentrations than requested by guideline: limit test, expected low toxicity
- Range finding study
- Test concentrations: --
- Results used to determine the conditions for the definitive study: --

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	96 h
Endpoint	LL0
Effect conc.	10000 mg/L
Nominal/Measured	nominal
Conc. based on	test mat.
Basis for effect	mortality : 0/20 animals died
Remarks (e.g. 95% CL)	

Details on results

- Behavioural abnormalities: none
- Observations on body length and weight: none
- Other biological observations: no particular findings
- Mortality of control: none
- Other adverse effects control: none
- Abnormal responses: none

Results with reference substance (positive control)

not appliicable

Reported statistics and error estimates

not applicable

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Test substance was not acutely toxic to Brachydanio rerio at a loading of 10,000 mg/l.

Executive summary

After 96 h of exposure all animals were alive and their condition (swimming behaviour, colour, respiratory function or any other visually observable morphological or behavioural criterion) was equal to that of the control animals.

6.1.2 Long-term toxicity to fish

6.1.3 Short-term toxicity to aquatic invertebrates

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

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	26 Sep. 1991 - 27 Sep. 1991
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Guideline study with acceptable restrictions (24 h instead of 48 h)		

Data source

Reference

Reference type	study report		
Author	Hooftman RN, van Drongelen-Sevenhuijsen D	**Year**	1992
Title	The acute toxicity to Daphnia magna (OECD guideline 202, 24 h)		
Bibliographic source	Unpublished		
Testing laboratory	TNO Institute of Environmental Sciences, Delft/NL	**Report no.**	IMW-91-0050-01
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 92-0139-DGO	**Report date**	1992-09-23

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Test guideline

Qualifier according to

Guideline OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)

Deviations yes Test duration 24 h (acc. to the valid guideline of 04 April 1984) instead of 48 h (today) / In one test, the oxygen content was 4.4 mg/L at time 0, i.e. less than 60 % of saturation (not assumed to have affected the outcome).

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

approx. 99.8 % (SiO_2): CAS-Name: Silica, amorphous,fumed, cryst.-free; CAS-No.: 112945-52-5

Analytical monitoring

no

Vehicle

no

Details on test solutions

PREPARATION AND APPLICATION OF TEST SOLUTION (especially for difficult test substances)
- Method: stirring for 20 h at 20 °C, followed by gross filtration (perlon wool) and microfiltration (1.7 µm and/or 1.2 µm)
- Eluate: no, filtrate Filtration: through perlon wool, then through glass-fibre filter A filtrate through 1.7 microfilter (still milky/cloudy)B. filtrate through 1.7 and 1.2 µm microfilter (visibly clear)
- Differential loading: no, limit test with 1000 mg/L
- Controls: Synthetic medium from groundwater
Note: In two pre-tests, suspensions with 1000 and 10,000 mg/L SiO_2 were used.

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

TEST ORGANISM
- Common name: see above
- Strain: --
- Source: laboratory culture
- Age at study initiation (mean and range, SD): <24 h
- Method of breeding: standard conditions according to principles of NPR 6503 (Nederlandse practijkrichtlijn of 1980)
- Feeding during test: no

Study design

Test type

static

Water media type

freshwater

Limit test

yes

Total exposure duration

24 h

Remarks

Post exposure observation period

none

Test conditions

Hardness

204 mg/L as $CaCO_3$

Test temperature

20 ± 1°C

pH

7.9 - 8.2

Dissolved oxygen

4.4 - 8.3 mg/L

Salinity

not applicable

Nominal and measured concentrations

1000 mg/L SiO_2
nominal

Details on test conditions

TEST SYSTEM
- Test vessel: 150-ml beaker
- Type: open - Material, size, headspace, fill volume: glass, 50 mL headspace, 100 mL test volume
- Aeration: none
- Renewal rate of test solution (frequency/flow rate): none
- No. of organisms per vessel: 5
- No. of vessels per concentration (replicates): 8
- No. of vessels per pure control (replicates): 8 (synthetic medium)
- No. of vessels per filtration control (replicates): 8 (test in synthetic medium passed through perlon filter)
- Biomass loading rate: 5 animals/100 mL

TEST MEDIUM / WATER PARAMETERS
- Source/preparation of dilution water: groundwater with mineral salts supplemented (synthetic medium)
- Total organic carbon: 1.5 mg/L
- Alkalinity: no data
- Ca/mg ratio: 1.76
- Conductivity: no data

OTHER TEST CONDITIONS
- Adjustment of pH: no
- Photoperiod: 16 h light, 8 h dark
- Light intensity: no data

Reference substance (positive control)

no

Results and discussions

Effect concentrations

Duration	24 h		
Endpoint	EL50		
Effect conc.	> 1000 mg/L		
Nominal/Measured	nominal		
Conc. based on	test mat.	**Basis for effect**	mobility : 1/40 animals were immobile (see Results table above)
Remarks (e.g. 95% CL)			

Duration	24 h
Endpoint	EL0
Effect conc.	1000 mg/L
Nominal/Measured	nominal

Conc. based on	test mat.	**Basis for effect**	mobility : 1/40 animals were immobile (see Results table above)

Remarks (e.g. 95% CL)

Details on results

- Mortality/immobility of control: none [0/40 animals]
- Mortality/immobility of treated animals:

1/40 Overall, 1/40 animals treated with the WAFs was found immobile after 24 h of exposure (2.5 %) (Report, Table B5 and B6 [Third (main) test]:
This consisted of two parallel series using clear or slightly milky solutions of the water-soluble fractions (WSF):
0/15 immobile animals (0 %) (assumed to relate to test medium microfiltrated 1.7 µm
#)1/25 immobile animals (4 %) (in the clear solution, assumed to relate to test medium microfiltrated 1.7 µm and 1.2 µm
#)- Abnormal responses: Based on pre-tests with suspensions, it is suspected that the immobility observed, particularly with the 10,000 mg/l suspensions, could be attributed to physical effects.
Note: The wording under 3.5 (p. 14) says that "....there was one immobile animal (out of 25) in the clear solution prepared by filtration of the 1000 mg/L solution". This statement would correlate with the test medium microfiltrated at 1.7 and 1.2 µm, according to Report 3.4.3 (p. 14). However, the allocation of the results to the filtration conditions is inconsistent due to contradictory indexing in the Tables B5 and B6 of the Report (p.24/25).

Results with reference substance

not applicable

Reported statistics and error estimates

not applicable

Remarks on results including tables and figures

It is not possible to include tables. Therefore attached as background material (table 1).

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

Test substance is not acutely toxic at a loading of ≥1 000 mg/l.

6.2 Sediment toxicity

6.3 Terrestrial toxicity

6.4 Biological effects monitoring

6.5 Biotransformation and kinetics

6.6 Additional ecotoxicological

7. TOXICOLOGICAL INFORMATION

7.1 Toxicokinetics, metabolism and distribution

7.1.1 Basic toxicokinetics

Endpoint study record: Basic toxicokinetics.001

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability Acceptable well documented study report which meets basic scientific principles

Data source

Reference

Reference type	study report		
Author	Reuzel PGJ, Woutersen RA, Bruijntjes JP	**Year**	1987
Title	Subchronic (13-week) inhalation toxicity study of aerosols of test substance and quartz in rats		
Bibliographic source	Unpublished report		
Testing laboratory	TNO Division of Nutrition and Food Research, Zeist/NL	**Report no.**	V 86.347/240718
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 87-0004-DGT	**Report date**	1987-05-14

Data access

other: Data owner or letter of access

Data protection claimed

yes, but willing to share

Cross-reference to same study

Materials and methods

Type of method

in vivo

Objective of study

other: deposition and clearance of SiO2 from lung

Test guideline

Qualifier equivalent or similar to

Guideline other guideline: OECD 413

Deviations yes Special modifications as compared with standard study: Focus upon lung, respiratory tract, and regional lymph nodes. Post-exposure recovery period up to one year.

Principles of method if other than guideline

Measurements of Si in lung and lymph nodes within repeated-dose toxicity study: Analytical method for silica determination (Report, part 1, p. 25): Lung and lymph node tissue were ashed according to the temperature program up to 650 °C in a platinum crucible. Following this, the ash was dissolved in 10 % hydrogen fluoride for 30 min. at 50 °C, and a saturated boric acid solution (silicon standard solution, 1 mg/ml) was added. The Si content of the solution was determined using a Varian ASS flame atomic absorption spectrometer.

GLP compliance

yes

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Radiolabelling

no

Test materials

Confidential details on test material

- CAS-No.: 112945-52-5, CAS-Name: Silica, amorphous, fumed, crystalline-free
- Substance type: inorganic
- Physical state: solid- Surface area (BET): 151 m^2/g (Report p. 59 Specification Certificate)
- Analytical purity: >99.8 % (SiO_2)
- Particle size: The range of the geometric agglomerate/aggregate size distribution was 1 to about 120 µm for the amorphous silicas with a maxima at approx. 10 µm and 100 µm (Report 1987, p. 13)
- Stability under test conditions: stable
- Storage condition of test material: room temperature

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Central Institute for Breeding of Laboratory Animals TNO, Zeist/NL
- Age at study initiation: 4 weeks
- Weight at study initiation: 50 - 70 g
- Fasting period before study: no
- Housing: single during exposure
- Diet: no access during exposure
- Water: no access during exposure
- Acclimation period: 10 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ± 2
- Humidity (%): 50 – 70
- Air changes (per hr): 12x/h
- Photoperiod (hrs dark / hrs light): no data

Administration / exposure

Route of administration

inhalation

Details on exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: stainless steel exposure chamber, multitiered (manufactered by Hazelton)
- Exposure chamber volume: 2.3 m^3
- Method of holding animals in test chamber: single
- Exposure type: whole body
- Source and rate of air: Aerosol entrance at top of the chamber
- Method of conditioning air: no data
- System of generating particulates/aerosols: Institute´s dust generator with compressed air operating atomizer
- Temperature, humidity, pressure in air chamber: av. 21 - 23 °C, minimum 19.1, max. 25.4 °C / 65
- 75 % rel. humidity, during extreme weather occasionally up to 95.5 % or down to 48 %.
- Air flow rate: approx. 40 m^3/h
- Air change rate: 40 / 2.3 = ~17/h
- Method of particle size determination: due to electrostatic charge of the particles not measured: technical failure of the 10-stage Mercer cascade impactor and the QCM cascade (Report p. 16)
- Treatment of exhaust air: filtered before release

TEST ATMOSPHERE
- Brief description of analytical method used: gravimetrically
- Air samples are drawn through glass fiber filters (Sartorius) and weighed (3 - 4 time per day)- Samples taken from breathing zone: no data

Duration and frequency of treatment / exposure

90 day(s)

Doses / concentrations

1.3, 5.9 or 31 mg/m3 (mean analytical values)

No. of animals per sex per dose

10 each after exposure (13 weeks) and recovery period (1, 13, 29, 39, and 52 wks): i.e. 50 m / 50 f animals per group were kept for a recovery period of at most 52 wks

Control animals

yes, concurrent no treatment

Positive control

no, but comparative study also including quartz

Details on study design

- Dose selection rationale: see 7.5.3

Details on dosing and sampling

PHARMACOKINETIC STUDY (Absorption, distribution, excretion) of SiO_2
- Tissues and body fluids sampled: lung and mediastinal lymph nodes
- Time and frequency of sampling: 1, 13, 29, 39, and 52 weeks post exposure, 10 animals each)

Statistics

The statistical assessment of the findings for the different parameters considered was based on analysis of variance (ANOVA) and Dunnett´s test

Results and discussions

Pharmacokinetic studies

Toxicokinetic parameters

Test No. #1 Half-life 1st: rapid from lung of male rats: not determinable by means of and at selected intervals (see Table below).

Test No. #2 Half-life 2nd: rapid from lung of female rats: not determinable by means of and at selected intervals (see Table below).

Metabolite characterisation studies

Metabolites identified

not measured

Overall remarks, attachments

Overall remarks

SILICA DEPOSITION

Silica could be detected in lungs only in relatively small amounts one week after the end of the exposure period, on the average 0.2 mg in all animals of the 30-mg groups, in 10 male and 7 female rats of the 6-mg groups, and in 3 animals of each in the 1-mg groups. Only in one untreated male animal, a low level of Si was detected. Only one male exposed to 30 mg/m^3 showed a small amount of silica in the regional lymph node. No significant increased Si levels were observed at any other recovery interval.

Applicant's summary and conclusion

Interpretation of results

no bioaccumulation potential based on study results

Endpoint study record: Basic toxicokinetics_NM 203_Gavage

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	W De Jong	**Year**	2013
Title	Deliverable 7: Identification of target organs and biodistribution including ADME parameters		
Bibliographic source			
Testing laboratory	ISS (I)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: Normal saline (NaCl 0.90% w/v).

Duration and frequency of treatment / exposure

Administration: repeated (on 5 consecutive days, day 1-5) Sampling time: day 6 and day 14

Doses / concentrations

20 mg/kg bw (male and female) per aministration. Cumulative dose: 100 mg/kg bw

No. of animals per sex per dose

Treated Groups: 3 M + 3 F Vehicle control: 3 M + 3 F

Details on dosing and sampling

Tissues Sampled: liver, spleen, GI tract (small intestine), mesenteric lymphnodes

Overall remarks, attachments

Attached full study report

NGTX_Toxicokinetics Metabolism Distribution_NM203_Gavage_ISS.docx / 17.82 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other: Very low levels in the liver and spleen (< 2 mg/kg organ weight) near the LOQ and LOD indicating a very low absorption from the gastro-intestinal tract.

Conclusions

Bioaccumulation negligible or absent of NM203 following repeated oral administration of 20 mg/kg

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Basic toxicokinetics_NM 203_IV

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	W De Jong	**Year**	2013
Title	Deliverable 7: Identification of target organs and biodistribution including ADME parameters		
Bibliographic source			
Testing laboratory	ISS (I)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed
yes, but willing to share

Materials and methods

Type of method
in vivo

Reference Material/Nanomaterial and Sample identification number
Identifier Reference Material/Nanomaterial

Identity NM 203

Test animals
Species
rat

Strain
Sprague-Dawley

Sex

male/female

Administration / exposure

Route of administration
intravenous

Vehicle
other: Normal saline (NaCl 0.90% w/v)

Duration and frequency of treatment / exposure
Administration: Single (day 1) or repeated (on 5 consecutive days, day 1-5)
Sampling time:
- Single admin: day 2 and day 90
- Repeated admin: day 6, 14, 30 and 90 (day 6 and 90 for female)

Doses / concentrations
20 mg/kg bw (male and female) per aministration. Cumulative dose: 100 mg/kg bw

No. of animals per sex per dose
Treated Groups: 3 M + 3 FControl: vehicle 3 M + 3 F

Details on dosing and sampling

liver, spleen, kidneys, heart, lungs, brain, testes/ovaries

Overall remarks, attachments

Attached full study report
NGTX_Toxicokinetics Metabolism Distribution_NM203_IV_ISS.docx / 22.46 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other: Single administration: At day 6, highest concentrations of Si were detected in spleen >liver, lungs and levels of Si above background in heart, kidney, testis. At day 90 level of Si still higher than control in spleen and liver.

Conclusions

Spleen and liver granuloma at day 90

Cross-reference to other study

http://www.nanogenotox.eu/

7.2 Acute Toxicity

7.2.1 Acute toxicity: oral

Endpoint study record: Acute toxicity: oral.001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	1977
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Basic data given, comparable to guideline study		

Data source

Reference

Reference type	study report		
Author	Leuschner F	**Year**	1977
Title	Pruefung der akuten Toxizität an Sprague-Dawley-Ratten bei peroraler Applikation		
Bibliographic source	unpublished report		
Testing laboratory	Laboratorium für Pharmakologie und Toxikologie (LPT), Hamburg	**Report no.**	
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 77-0004-DKT	**Report date**	1977-12-28

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Test type

standard acute method

Test guideline

Qualifier no guideline available

Guideline

Deviations not applicable

Qualifier equivalent or similar to

Guideline OECD Guideline 401 (Acute Oral Toxicity)

Deviations

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

>98 % (SiO2): CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: S. Ivanovas/Kißlegg, Germany
- Age at study initiation: 38 d (male), 42 d (female)
- Weight at study initiation: 100 - 105 g
- Fasting period before study: 15 - 16 h before start of the study
- Housing: single in Macrolon cages

- Water: ad libitum
- Acclimation period: no data

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 24 ± 0.5 °C
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): no data

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: water/methyl-hydoxyethyl cellulose 300 P (1%)

Details on oral exposure

VEHICLE
- Concentration in vehicle: no data
- Amount of vehicle (if gavage): no data
- Justification for choice of vehicle: stabilisation of homogeneous distribution of light insoluble test material in aqueous gel suspension

Doses

2000 and 3300 mg/kg bw.

No. of animals per sex per dose

10

Control animals

no

Details on study design

- Duration of observation period following administration: 28 days
- Frequency of observations and weighing: data on days 1, 2, and 14 (Tab. 2)
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight, food consumption mentioned

Statistics

not relevant

Results and discussions

Effect levels

Sex	male/female
Endpoint	LD50
Effect level	> 3300 mg/kg bw
95% CL	
Remarks	

Mortality
no mortality

Clinical signs
no particular findings

Body weight
slight reduction of 4 - 8 %, measured at days 1, 2, and 14

Gross pathology
no particular findings

Other findings
- Other observations: feed consumption reduced in the 2000-mg groups (10, 4 and 6 % at day 1, 2, and 14, respectively)

Applicant's summary and conclusion

Interpretation of results
other: non-toxic

Endpoint study record: Acute toxicity: oral.002

Administrative Data

Purpose flag	supporting study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	July 1974
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Test procedure in accordance with national standard methods with acceptable restrictions, Summary report		

Data source

Reference

Reference type	study report		
Author	Powers MB	**Year**	1964
Title	Acute oral administration to mice		
Bibliographic source	unpublished report		
Testing laboratory	Hazelton Laboratories, Virginia/USA	**Report no.**	
Owner company	Cabot Corporation		
Company study no.		**Report date**	1964-02-24

Data access
other: data owner or letter of access

Data protection claimed
yes, but willing to share

Materials and methods

Test type
standard acute method

Test guideline
Qualifier equivalent or similar to

Guideline OECD Guideline 401 (Acute Oral Toxicity)

Deviations

GLP compliance
no

Test materials

Test material equivalent to submission substance identity
yes

Test material identity
Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Identifier other:

Identity The test substance is equivalent to NM 203.

Details on test material
CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5

Confidential details on test material
The test substance is equivalent to NM 203.

Test animals

Species
mouse

Strain
Swiss

Sex
male

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Doses

178, 316, 562, 1000, 1780 and 3160 mg/kg

No. of animals per sex per dose

10

Any other information on materials and methods incl. tables

The test substance was given by gavage at variable volumes, at maximum 10 ml/kg.

Results and discussions

Effect levels

Sex	male
Endpoint	LD50
Effect level	> 3160 mg/kg bw
95% CL	
Remarks	

Remarks on results including tables and figures

No adverse signs of toxicity in any animal during the study, no macroscopic lesions upon necropsy after 14-d observation.

Applicant's summary and conclusion

Interpretation of results

other: non-toxic

7.2.2 Acute toxicity: inhalation

Endpoint study record: Acute toxicity: inhalation.001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	18 April - 02 May 1983
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Comparable to guideline study with acceptable restrictions (limited documentation)		

Data source

Reference

Reference type	study report		
Author	Appelman LM, Reuzel PGJ	**Year**	1983
Title	Acute inhalation toxicity study in rats		
Bibliographic source	Unpublished report		
Testing laboratory	TNO Division for Nutrition and Food Research, Zeist/NL	**Report no.**	V 83.142/221216
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 83-0016-DGT	**Report date**	1983-06-10

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Test type

standard acute method

Limit test

yes

Test guideline

Qualifier according to

Guideline OECD Guideline 403 (Acute Inhalation Toxicity)

Deviations yes The highest attainable exposure concentration was not applied due to technical limitations. Air exchange of the inhalation chamber was lower than recommended 0.8/h instead of 10 - 15/h.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

>98 % (SiO2): CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Central Institute for Breeding of Laboratory Animals TNO, Zeist/NL
- Weight at study initiation: 191 - 199 g: av. 194 g (male); 144 - 149 g: av. 147 g (female)
- Fasting period before study: no
- Housing: single during exposure
- Diet: ad libitum until start
- Water: ad libitum until start
- Acclimation period: no data

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21 ± 1
- Humidity (%): 50 - 60

Administration / exposure

Route of administration

inhalation: dust

Type of inhalation exposure

nose only

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: stainless steel exposure chamber provided with glass windows
- Exposure chamber volume: 1.5 m^3
- Method of holding animals in test chamber: single
- Source and rate of air: entrance near the pyramidal top; 1.2 m^3/h ==> exchange rate = 0.8/hour
- Method of conditioning air: no data
- System of generating particulates/aerosols: Dispersing the powder continuously by means of a "Buerstendosierer" Typ III/A
- Method of particle size determination: cascade impactor

TEST ATMOSPHERE
- Brief description of analytical method used: gravimetrically
- amount of dust on glass fiber filter divided by the amount of air applied (at 4 time point during exposure)

Nominal concentration calculated from the the total quantity of test material devided by the amount of air

applied

TEST ATMOSPHERE
- Particle size distribution: approx. 47 - 50 mass% ≤ 6 µmf rom Report Tab. 1:

Distribution aerodynamic in % of total weight	aerodynamic diameter (µm)
4.7	0.47
6.0	0.7
7.2	1.1
6.6	1.7
2.2	2.5
4.2	3.4
6.4	4.3
9.9	5.7
52.7	≥7.7

MMAD (Mass median aerodynamic diameter) / GSD (Geometric st. dev.): MMAD = ~3.2 µm / GSD: no data
Note: calculated from the VMD (Volume Mean Diameter) of 63.0 µm multiplied with the density of about 0.05 g/cm3.
CLASS METHOD (if applicable)- Rationale for the selection of the starting concentration: maximum attainable concentration

Analytical verification of test atmosphere concentrations

yes

Duration of exposure

4 h **Remarks**

Concentrations

maximum technically attainable analytical concentration: av. 139 mg/m^3 (range 110 - 190 mg/m^3)
Nominal concentration: 16.7 g/m^3

No. of animals per sex per dose

5

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: body weight on days 0, 2, 4, 7, and 14
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight

Statistics

not relevant

Any other information on materials and methods incl. tables

Nose-only exposure system. Five animals each per sex were used. Due to substance-inherent properties resulting in sedimentation and adsorption to the equipment, the maximum technically attainable aerosol concentration in the chamber ranged from 110 to 190 mg/m_3, while the nominal concentration was 16.7 g/m^3. About 47 % of the aerosol comprised particles with an aerodynamic diameter of <6 µm (part of

respirable fraction) [see above: "Details on inhalation exposure"].

Results and discussions

Effect levels

Sex	male/female
Endpoint	LC0
Effect level	≥ 0.14 mg/L air (analytical)
95% CL	
Exp. duration	4 h
Remarks	
Sex	male/female
Endpoint	LC50
Effect level	≥ 0.14 mg/L air (analytical)
95% CL	
Exp. duration	4 h
Remarks	

Mortality

none

Clinical signs

Restlessness, half-closed eyes

Body weight

slight decrease or stagnation on day 2, but not related to previous exposure (note: By mistake animals were deprived of water for 16 h directly after exposure.)

Gross pathology

no particular findings

Other findings

none

Remarks on results including tables and figures

No clinical symptoms and no findings at autopsy after 14 d post-treatment.

Applicant's summary and conclusion

Interpretation of results

other: none-toxic

Endpoint study record: Acute toxicity: inhalation.002

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 14 - 28 Sep. 1981

Reliability 1 (reliable without restriction)

Rationale for reliability Comparable to guideline study

Data source

Reference

Reference type	study report		
Author	Toxigenics	**Year**	1981
Title	Four hour acute dust inhalation toxicity study in rats		
Bibliographic source	Unpublished report		
Testing laboratory	Toxigenics Inc., Illinois/USA	**Report no.**	420-0690
Owner company	Cabot Corporation		
Company study no.		**Report date**	1981-10-12

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Test type

standard acute method

Limit test

yes

Test guideline

Qualifier equivalent or similar to

Guideline OECD Guideline 403 (Acute Inhalation Toxicity)

Deviations yes The highest attainable exposure concentration was limited for technical reasons.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Identifier other:

Identity The test substance is equivalent to NM 203.

Details on test material

CAS-Name: Silica, amorphous, fumed, crystalline-free; CAS-No.: 112945-52-5, purity ca. 100 %

Confidential details on test material

The test substance is equivalent to NM 203.

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Harlan Sprague Dawley, Madison/Wisconsin
- Age at study initiation: "young" (no further data)
- Weight at study initiation: 258 - 297 g (male); 215 - 240 g (female)
- Fasting period before study: no
- Housing: single
- Diet: ad libitum except during exposure
- Water: ad libitum except during exposure
- Acclimation period: ≥ 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): data not included in the report
- Humidity (%): data not included in the report
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): 12 / 12 hours

Administration / exposure

Route of administration

inhalation: dust

Type of inhalation exposure

whole body

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: stainless steel and glass inhalation chamber
- Exposure chamber volume: total 80 L with dimensions of the central unit of approx. 50 L [12 inch high, 18 inch wide, 18 inch deep (approx. 30 x 46 x 46 cm)]
- Method of holding animals in test chamber: as group in the chamber cage
- Source and rate of air: from the top center of the chamber, 56 - 85 L/min
- Method of conditioning air:
- System of generating particulates/aerosols: stream of air passed through the test material contained in a 3-L 3-necked flask equipped with a magnetic stirrer
- Method of particle size determination: using a Delron Cascade Impactor, model No. DCI-6: Sample collected a approx. one and 3 h into the exposure
- Treatment of exhaust air: collected at the bottom and relaease to outside after dilution
- Temperature, humidity, pressure in air chamber: mean 72.3 °F (room) / mean 76.6 °F (chamber)

TEST ATMOSPHERE
- Brief description of analytical method used: gravimetric
- amount of dust collected on glass fiber filter divided by air volume
- Samples taken from breathing zone: yes

TEST ATMOSPHERE (Report Tab. 2 and 3)
- Particle size distribution: based on sample 1 and 2 16 mass % ≤ 0.325 µm / 0.168 µm
50 mass% ≤ 0.989 µm / 0.534 µm
83 mass% ≤ 3.007 µm / 1.698 µm
98.13 and 99.43 mass% ≤ 10 µm
- MMAD (Mass median aerodynamic diameter) / GSD (Geometric st. dev.):
MMAD = 0.76 µm / GSD = 3.11

CLASS METHOD (if applicable)
- Rationale for the selection of the starting concentration: For technical limitations, the highest attainable concentration was applied.

Analytical verification of test atmosphere concentrations

yes

Duration of exposure

4 h **Remarks**

Concentrations

Analytical concentration: 2.08 mg/L (average of 10 samples with a range from 1.63 to 2.70 mg/L, one outlier with 0.45 mg/L) (Report Tab. 1)
Nominal concentration:58.8 mg/L

No. of animals per sex per dose

5

Control animals

yes

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: 2x/d / body weight on days 0, 7, and 14
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight

Any other information on materials and methods incl. tables

Whole-body exposure: A control group of ten rats exposed to clean air was run in parallel. Post-exposure observation 14 d. Air concentration was analysed by sampling dust on glass fiber filters and determining the sampled amount by gravimetry. Particle size distribution was measured using a Delron Cascade impactor (MMAD = 0.76 µm ± 3.11). Approx. 84 % of the particles had a diameter of ≤ 3 µm, approx. 98 % ≤ 10 um. All animals were subjected to gross necropsy.

Results and discussions

Effect levels

Sex	male/female
Endpoint	LC0
Effect level	≥ 2.08 mg/L air (analytical)
95% CL	
Exp. duration	4 h
Remarks	
Sex	male/female
Endpoint	LC50
Effect level	> 2.08 mg/L air (analytical)
95% CL	
Exp. duration	4 h
Remarks	
Sex	male/female
Endpoint	LC0
Effect level	≥ 58.8 mg/L air (nominal)
95% CL	
Exp. duration	4 h
Remarks	
Sex	male/female
Endpoint	LC50
Effect level	> 58.8 mg/L air (nominal)
95% CL	
Exp. duration	4 h
Remarks	

Mortality

no

Clinical signs

Nasal discharge, crusty eyes, alopecia, and crusty nose

Body weight

no particular findings, normal

Gross pathology

Discoloration of the lung in one test rat, no other particular findings

Remarks on results including tables and figures

No animals died. Nasal discharge during exposure, crusty eyes, crusty nose and alopecia at days post-exposure. No macroscopic organ lesions, but in one animal discoloration of the lung.

Applicant's summary and conclusion

Interpretation of results

other: none-toxic

7.3 Irritation / corrosion

7.3.1 Skin irritation / corrosion

Endpoint study record: Skin irritation / corrosion.001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	Nov. 1977
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Comparable to guideline study with acceptable restrictions (documentation limited)		

Data source

Reference

Reference type	study report		
Author	Leuschner F	**Year**	1978
Title	Lokale Vertraeglichkeit an der Kaninchenhaut (Patch-Test)		
Bibliographic source	Unpublished report		
Testing laboratory	Laboratorium für Pharmakologie und Toxikologie (LPT), Hamburg	**Report no.**	
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 78-0004-DKT	**Report date**	1978-03-01

Data access
other: data owner or letter of access

Data protection claimed
yes, but willing to share

Materials and methods
Type of method
in vivo

Test guideline
Qualifier equivalent or similar to

Guideline OECD Guideline 404 (Acute Dermal Irritation / Corrosion)

Deviations yes 24-h exposure + including abraded skin

Qualifier according to

Guideline other guideline: Patch-Test; Hazardous Substances, Part 191, Section 11, FDA, Washington, 1965

Deviations yes Exposure time 24 hours

GLP compliance
no

Test material equivalent to submission substance identity
yes

Test materials
Test material identity
Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material
CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5

Test animals
Species
rabbit

Strain
New Zealand White

Details on test animals and environmental conditions
TEST ANIMALS
- Weight at study initiation: 2.3 - 2.8 kg
- Housing: V2A steel cages

- Diet: ad libitum except treatment day
- Water: ad libitum except treatment day

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21 ± 2 °C
- Humidity (%): 60 ± 3
- Photoperiod (hrs dark / hrs light): 12 / 12 hrs

Test system

Type of coverage

occlusive

Preparation of test site

other: intact and abraded

Vehicle

other: 12-% suspension/gel in 1-% methyl-hydroxyethyl cellulose 300 P

Amount/concentration applied

Dose: 0.5 g

Duration of treatment / exposure

24 hour(s)

Observation period

14 days

Number of animals

6 (intact skin)6 (abraded skin)

Control animals

no

Details on study design

TEST SITE
- Area of exposure: approx. 2.5 cm x 2.5 cm
- Type of wrap if used: plasic foil

REMOVAL OF TEST SUBSTANCE
- Time after start of exposure: 24 h

SCORING SYSTEM:
Draize scoring scheme for grading skin effects (erythema and oedema)
Time: 24 and 72 h (0 and 48 h after termination of exposure)

Results and discussions

Irritation / corrosion results

Irritation parameter	primary dermal irritation index (PDII)
Basis	mean
Time point	24 + 72 h

Score 0

Max. score 8

Reversibility

Remarks

Irritant/corrosive response data

There were no signs of irritation.

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Conclusions

No classification (EU and GHS)

7.3.2 Eye irritation

Endpoint study record: Eye irritation.001

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** March 1978

Reliability 2 (reliable with restrictions)

Rationale for reliability Comparable to guideline study with acceptable restictions (limited documentation)

Data source

Reference

Reference type	study report		
Author	Leuschner F	**Year**	1978
Title	Schleimhautvertraeglichkeit am Kaninchenauge bei einmaliger Applikation		
Bibliographic source	Unpublished report		
Testing laboratory	Laboratorium für Pharmakologie und Toxikologie (LPT), Hamburg	**Report no.**	
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 78-0005-DKT	**Report date**	1978-07-20

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Test guideline

Qualifier according to

Guideline other guideline: Draize-Test; Hazardous Substances, Part 191, Section 12, Federal Register, Vol. 37, No. 83, FDA, Washington

Deviations

GLP compliance

no

Test material equivalent to submission substance identity

yes

Test materials

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS
- Source: no data
- Age at study initiation: no data
- Weight at study initiation: 2.3 - 2.8 kg
- Housing: V2A steel cages
- Diet: ad libitum except treatment day
- Water: ad libitum except treatment day
- Acclimation period: no data

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21 ± 2 °C
- Humidity (%): 60 ± 3
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): 12 / 12 hrs

Test system

Vehicle

unchanged (no vehicle)

Amount/concentration applied

Dose: 100 mg

Duration of treatment / exposure

24 h, not rinsed

Observation period

96 hours, if necessary prolonged

Number of animals

3

Control animals

not required

Details on study design

The substance was applied into the conjunctival sac of the left eye, while the right eye remained untreated serving as control.

Results and discussions

Irritant/corrosive response data

No irritating response at any time after exposure (24 - 96 h).

Remarks on results including tables and figures

No irritating response at any time after exposure (24 - 96 h).

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Conclusions

No classification (EU and GHS)

7.4 Sensitisation

7.5 Repeated dose toxicity

7.5.1 Repeated dose toxicity: oral

7.5.2 Repeated dose toxicity: inhalation

7.5.3 Repeated dose toxicity: inhalation

Endpoint study record: Repeated dose toxicity: inhalation.001

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	Exposure: 20 Jul. 1984 - 19 Oct. 1984 / end observation: 15 Oct. 1985
Reliability	1 (reliable without restriction)		
Rationale for reliability	GLP guideline study		

Data source

Reference

Reference type	study report		
Author	Reuzel PGJ, Woutersen RA, Bruijntjes JP	**Year**	1987
Title	Subchronic (13-week) inhalation toxicity study of aerosols and quartz in rats		
Bibliographic source	Unpublished report		
Testing laboratory	TNO Division for Nutrition and Food Research, Zeist/NL	**Report no.**	V 86.347/240718
Owner company	Evonik Industries AG		
Company study no.	Degussa AG - US-IT-No. 87-0004-DGT	**Report date**	1987-05-14

Reference type	publication		
Author	Reuzel PGJ, Bruijntjes JP, Feron VJ, Woutersen RA	**Year**	1991
Title	Subchronic inhalation toxicity of amorphous silicas and quartz dust in rats		
Bibliographic source	Fd. Chem. Toxicol., 29, 341-354		
Testing laboratory	TNO Toxicology and Nutrition Institute, Zeist/NL	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other:

Data protection claimed

yes

Materials and methods

Test type
subchronic

Limit test
no

Test guideline
Qualifier equivalent or similar to

Guideline OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)

Deviations yes Special modifications as compared with standard study: Focus upon lung, respiratory tract, and regional lymph nodes. Post-exposure recovery period up to one year.

Principles of method if other than guideline
Comparative study including test substance as well as quartz (crystalline).

GLP compliance
yes

Test materials
Test material equivalent to submission substance identity
yes

Test material identity
Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material
- CAS-Name: Silica, amorphous, fumed, crystalline-free, CAS-No.: 112945-52-5
- Substance type: inorganic
- Physical state: solid
- Surface area (BET): 151 m^2/g (Report p. 59 Specification Certificate)
- Analytical purity: >99.8 % (SiO_2)
- Particle size: The range of the geometric agglomerate/aggregate size distribution was 1 to about 120 µm for the amorphous silicas with maxima at approx. 10 µm and 100 µm (Report 1987, p. 13)
- Stability under test conditions: stable
- Storage condition of test material: room temperature

Test animals
Species
rat

Strain
Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Central Institute for Breeding of Laboratory Animals TNO, Zeist/NL
- Age at study initiation: 4 weeks
- Weight at study initiation: 50 - 70 g
- Fasting period before study: no
- Housing: single during exposure-
Diet: no access during exposure
- Water: no access during exposure
- Acclimation period: 10 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ± 1
- Humidity (%): 50 - 70
- Air changes (per hr): 12x/h
- Photoperiod (hrs dark / hrs light): no data

Administration / exposure

Route of administration

inhalation

Type of inhalation exposure

whole body

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: stainless steel exposure chamber, multitiered (manufactered by Hazelton)
- Exposure chamber volume: 2.3 m^3
- Method of holding animals in test chamber: single
- Source and rate of air: Aerosol entrance at top of the chamber
- Method of conditioning air: no data
- System of generating particulates/aerosols: Institute´s dust generator with compressed air operating atomizer
- Temperature, humidity, pressure in air chamber: av. 21 - 23 °C, minimum 19.1, max. 25.4 °C / 65
- 75 % rel. humidity, during extreme weather occasionally up to 95.5 % or down to 48 %.
- Air flow rate: approx. 40 m^3/h
- Air change rate: 40 / 2.3 = ~17/h
- Method of particle size determination: due to electrostatic charge of the particles not measured: technical failure of the 10-stage Mercer cascade impactor and the QCM cascade (Report p. 16)
- Treatment of exhaust air: filtered before release

TEST ATMOSPHERE
- Brief description of analytical method used: gravimetrically
- Air samples are drawn through glass fiber filters (Sartorius) and weighed (3 - 4 times per day)
- Samples taken from breathing zone: no data

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

see Report Tables (Part 2), Table 1:
Daily mean concentrations are documented:based on 250 - 253 measurements:
1.26 (SEM 0.08) mg/m^3; 5.88 (SEM 0.88) mg/m^3; 31.04 (SEM 0.87) mg/m^3

Duration of treatment / exposure

13 weeks

Frequency of treatment

6 hours/day, 5 days/week

Doses/concentrations

1.3, 5.9 or 31 mg/m^3 (mean analytical values)

Basis analytical conc.

1, 6 and 30 mg/m^3 (target concentrations)

Basis nominal conc.

MMAD / GSD

no monitoring data due to technical difficulties (see above "Details on inhalation exposure")

No. of animals per sex per dose

70 test substance: assigned dose groups B, C, and D, each sub-divided in 7 sub-groups a, b, c , d, e, f, and g; 10 each (sacrificed after 13 wks), 50 each kept for a recovery period of at most 52 wks (13, 26, 39, and 52 wks).

Control animals

yes

Details on study design

- Dose selection rationale: based on range findings (14 d)
- Rationale for selecting satellite groups: post-exposure recovery period for examination of reversibility of effects
- Post-exposure recovery period in satellite groups: 13, 26, 39, and 52 wks

Positive control

Quartz (crystalline silica, 58 mg/m3) included (assigned Group G)

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: yes
- Time schedule: 2x/day, 1x/d (weekends)
- Cage side observations checked in table 3 and 4 (mortalities) were included.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: see body weight

BODY WEIGHT: Yes

- Time schedule for examinations: start, weekly during exposure, 1x/wk during recovery
- Tables 5 and 6
FOOD CONSUMPTION:
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: No data

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

WATER CONSUMPTION: No data
- Time schedule for examinations:

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: week 13, 26, 39, 52, 65 (i.e. including recovery period)
- Anaesthetic used for blood collection: No (data)
- Animals fasted: No data
- How many animals: 10 males, 10 females
- Tables 7-16

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: week 14, 27, 40, 53, and 66
- Animals fasted: Yes overnight
- How many animals: 10 males, 10 females
- Parameters in tables 17 - 26

URINALYSIS: Yes
- Time schedule for collection of urine: week 13, 26, 40/41, 52, and 65
- Animals fasted: Yes
- Parameters in tables 27 – 36

NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes (Table 63 - 67) Relative organ weights (Table 37 - 56)
HISTOPATHOLOGY: Yes (see table 68 - 73), in particular lung and lymph nodesin addition:
Si contents of lung and lymph nodes (Tables 59 - 62)
Collagen content in lung (Tables 57/58)

Other examinations

Relative organ weights (Table 37 - 56)

Statistics

Body weights: analysis of co-variance followed by Dunnett´s test
Histopathological changes and mortality: Fisher´s exact probability test
Organ weights, blood parameter: analysis of variance and Dunnett´s test

Results and discussions

Effect levels

Endpoint	NOAEC
Effect level	1.3 mg/m³ air (analytical)
Sex	male/female
Basis for effect level / Remarks	Histopathology: based on the slight and fully reversible pulmonary response noted at this exposure level (inflammation reaction) (see details below "Details on results")
Endpoint	LOAEC
Effect level	5.9 mg/m³ air (analytical)
Sex	male/female
Basis for effect level / Remarks	(haematology); organ weights (hypertrophy lung); histopathology (collagen increase, sporadic focal fibrosis)
Endpoint	NOEC
Effect level	< 1.3 mg/m³ air (analytical)
Sex	male/female
Basis for effect level / Remarks	Histopathology: based on the pulmonary response (inflammation reaction) (see details below "Details on results")

Observations

Clinical signs and mortality

yes

Body weight and weight gain

yes

Food consumption

no data

Food efficiency

no data

Water consumption

no data

Ophthalmoscopic examination

not examined

Haematology

yes

Clinical chemistry
yes

Urinalysis
yes

Neurobehaviour
not examined

Organ weights
yes

Gross pathology
yes

Histopathology: non-neoplastic
yes

Histopathology: neoplastic
yes

Details on results
CLINICAL SIGNS AND MORTALITY
Respiration rate: concentration-related increase No mortality

BODY WEIGHT AND WEIGHT GAIN
No effect in females at all dose levels (Tab. 6)
Depressive effect in males:
1 mg/m^3: slightly at day 14 of exposure only (~ -5%)
6 mg/m^3: slightly from day 49 to day 77 of exposure (~ - 6 to <5 %) no more significant by end of exposure (day 91)
30 mg/m^3: significantly throughout exposure: ~ -7 - -10 %, day 91: -7 % Recovery: no difference from control at day 455 (52 weeks post-exposure)

HAEMATOLOGY
1 mg/m^3: no effects
6 mg/m^3: White blood cell count elevated in both males and females due to increases in the numbers of neutrophilic leukocytes,but concentration-response relationship was poor. After 3 months recovery, these blood parameters normalized in males and females.
30 mg/m^3: Red blood cell count and hemoglobin were statistically higher in males, but not in females. White blood cell count elevated in both males and females due to increases in the numbers of neutrophilic leukocytes, at 3 months of recovery (days 176/177, Table 8/Table 13), but concentration-response relationship was poor. In females, a slight increase above the control group apparently still existed after 6 months of recovery (day 275, Table 14).

CLINICAL CHEMISTRY
no significant effects

URINALYSIS
no significant effects

ORGAN WEIGHTS
No changes in heart, thyroid, thymus, adrenals, testes, brain, spleen, kidney
Treatment-related degrees of severity: swollen lungs and enlarged mediastinal lypmph nodes at the end of exposure

LUNG
1 mg/m^3: no significant increase
6 mg/m^3: mean increase in relative weight 1.7x (males), 1.4x (females)
30 mg/m^3: mean increase in relative weight 2.3x (males), 2.0x (females)

LYMPH NODE:
no weight data

PATHOLOGY
Swollen and spotted lungs and enlarged mediastinal lymph nodes, the degree of severity being treatment-related. At 6 and 30 mg/m^3, collagen content in the lungs was clearly increased, most pronounced in males. The above-mentioned effects gradually subsided after the exposure period, but in males exposed to 6 and 30 mg/m^3 the collagen content was still above control values at the end of the study.

HISTOPATHOLOGY: NON-NEOPLASTIC
Accumulation of alveolar macrophages and granular material, cellular debris, polymorphonuclear leucocytes, increased septal cellularity. Alveolar bronchialisation, focal interstitial fibrosis, cholesterol clefts and granuloma-like lesions in the lung. The granuloma-like lesions were seen in a few animals at the end of exposure period and after 13 weeks of recovery. They did not show fibroblastic activity and hyalinization and regressed during recovery [not progressive, i.e. no silicogenic nodules formed (no silicosis)]. Accumulation of macrophages was seen in the mediastinal lymph nodes (disappeared after wk 39 post-exposure). Treatment-related microscopic changes in the nasal region were occasionally found at the end of exposure period, such as focal necrosis and slight atrophy of the olfactory epithelium. Interstitial fibrosis was not noted directly after the exposure period, but appeared with a delay, for the first time observed after 13 wks post-exposure: increasing incidence especially in 30-mg rats, and a few in the 6-mg group (Report p. 44), but decreased in severity and frequency until the end of the study (Report p. 51).All types of pulmonary lesions were more marked in males than in females. The level of 1.3 mg/m^3 induced only slight changes after 13-wk exposure (Table 68), which generally recovered quickly (no differences from control after 13-wk post-exposure: Table 69). Morphological changes after 13-wk exposure, that were considered statistically significant at 1.3 mg/m^3 (Table 68):

	Male	Female	Male	Male
	Treated		**untreated**	
Accumulation of alveolar macrophages	slight in 10/10	(very) slight in 10/10	(very) slight 4/10	slight in 1/10
Intra-alveolar polymorphonuclear leukocyte	(very) slight in 6/10	(very) slight in 8/10	0/10	0/10
Increased septal cellularity	(very) slight in 10/10	(very) slight in 9/10	very slight in 1/10	very slight in 1/10
Olfactory epithelial atrophy	(very) slight in 5/10	(very) slight in 8/10	0/10	0/10
Intracytoplasmic proteinaceous droplets -respiratory epithelium	In 8/10	In 9/10	In 1/10	0/10
Mediastinal lymph node -macrophage accumulation:	(very) slight in 8/10	(very) slight in 8/10	0/10	0/10

HISTOPATHOLOGY: NEOPLASTIC
No particular findings

HISTORICAL CONTROL DATA (if applicable):
no data

OTHER FINDINGS

SILICA DEPOSITION
Silica could be detected in lungs only in relatively small amounts at the end of the exposure period (Tables 59): on the average 0.1 - 0.2 mg per lung of male animal groups (not dose-related), 0.05 - 0.21 mg per lung of female groups (dose-related). Only one male exposed to 30 mg/m^3 showed a small amount of silica in the regional lymph node. 90 days after termination of exposure (day 188), no silica could be recovered from any animal. (see Chapter 7.1: Degussa 87-0004-DGT_ inhal_Si-deposition, 13 wk, rat_key_RL2)

Overall remarks, attachments

Overall remarks

Inhaled amorphous silica provokes an inflammatory response in the respiratory tract of rats, in particular the lung, at low concentration. A progression process of any lesion was not observed like that seen after quartz exposure, i.e. all observations suggest reversibility, although rather slow except at the lowest exposure level. All synthetic amorphous silica was completely cleared from the lung, but clearance is different for various silica (see also other entries): for test substance very quickly. The granuloma-like lesions were not progressive, i.e. no silicogenic nodules formed (no silicosis). Survival was not affected in any of the groups. The only clinical sign noted with test substance was increased respiration rate. At the 1.3 mg-level, the effects were mild, completely cured after 13 wks recovery. There were no histologically manifested tissue changes. Therefore, this exposure concentration is considered the NOAEC for subchronic exposure, but depending on the pathological relevance placed on the observed effects, this NOAEC may be applicable to chronic exposure.

Endpoint study record: Repeated dose toxicity: inhalation.002

Administrative Data

Purpose flag supporting study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** Dec. 2001 - Aug. 2002

Reliability 1 (reliable without restriction)

Rationale for reliability GLP guideline study: Comparative study including three synthetic amorphous silicas

Data source

Reference

Reference type	study report		
Author	Arts JHE, Kuper CF	**Year**	2003
Title	A repeated 5-day inhalation study in rats, including two recovery periods, with synthetic amorphous silicas		
Bibliographic source	unpublished study		
Testing laboratory	TNO Chemistry, Zeist/NL	**Report no.**	V 4306
Owner company	Association of Synthetic Amorphous Silica Producers (ASASP/CEFIC)		
Company study no.	Degussa Ag - Nr. 2003 - 0111 - FGT	**Report date**	2003-12-03

Data access

other: data owner or letter of access

Data protection claimed

yes

Materials and methods

Test type

subacute

Test guideline

Qualifier equivalent or similar to

Guideline OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)

Deviations yes only 5 exposure days; only one sex (male); untreated control: only 6 animals; histopathology and organotoxicology limited; no clinical chemistry + haematology, but lung lavage cytology + biochemistry instead

Principles of method if other than guideline

Method: in accordance with OECD Guide-line 412, 12 May 1981 and directive 92/69/EEC, 29 Dec. 1992, but focus on the respiratory tract (lung and lymph nodes)
Test substance was examined only in males because they had proven to be more sensitive than females, as observed in the first study

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Identifier other:

Identity The test substance is equivalent to NM 203.

Details on test material

CAS-Name: Silica, amorphous, fumed, crystalline-free; CAS-No.: 112945-52-5, purity ca. 100 %

Confidential details on test material

The test substance is equivalent to NM 203.

Test animals

Species

rat

Strain

Wistar

Sex

male

Administration / exposure

Route of administration

inhalation

Type of inhalation exposure

nose only

Details on inhalation exposure

AEROSOL GENERATION:
Miniature screw conveyor, a dust feeder, (Institute´s design) connected to a low-velocity eductor in which the test material was aerolised. The eductors were operated with compressed humidified air. The test material was aerosolised and diluted with a defined amount of humidified air at the entrance of each exposure unit

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

EXPOSURE LEVELS and PARTICLE SIZE:
Mean actual concentrations: 1.39 (± 0.15), 5.41 (± 0.34), and 25.3 (± 0.9) mg/m^3.

Duration of treatment / exposure

5 days

Frequency of treatment

6 h/d

Doses/concentrations

1, 5, 25 mg/m^3

Basis nominal conc.

MMAD / GSD

Mass median aerodynamic diameter of particle size distribution (MMAD) = 1.2 - 1.3 µm or 2.2 - 3.5 µm (depending on the technical device used: see p. 20).[Note: This particle size distribution is artificial and experimentally produced, but the commercial product has a mean particle size of about 100 µm due to agglomeration of primary particles.]

No. of animals per sex per dose

10 males additionally, satellite groups of 10 males each were exposed correspondingly and kept for a recovery period of one and three months.

Control animals

yes, concurrent no treatment

Details on study design

Post-exposure period: 1 or 3 months

Positive control

One extra group was exposed to 25 mg/m^3 crystalline silica as a positive control group, included in study.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes

DETAILED CLINICAL OBSERVATIONS: Yes

BODY WEIGHT: Yes

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

FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: Yes

WATER CONSUMPTION: No data

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

CLINICAL CHEMISTRY: No

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No

OTHER: CYTOLOGY on LUNG CELLS in LAVAGE
At necropsy, 5 animals per group and sex were lavaged acc. to standard procedure. The lavage was used for white blood cell count, viability and cell differentiation (eosinophils, neutrophils, lymphocytes, monocytes/ macrophages, viable cells). The supernatant of the lavage was used for determination of biochemical parameters (total protein, albumin, ALP, LDH, N-acetyl glucosaminidase (NAG), SOD, GSH, and TNF-alpha).

SILICON CONTENT
Si content of the lung and tracheobronchial lymph nodes were determined. HYDROXY PROLINE

CONTENT
The OH-proline of the lung and tracheobronchial lymph nodes were determined.

Sacrifice and pathology

GROSS PATHOLOGY: Yes

HISTOPATHOLOGY:
Yes, but only kidney, lung and lymphnodes

Other examinations

See above "Observations and examinations..."

Statistics

Various procedures acc. to the parameters under test (Report, p. 19)

Results and discussions

Effect levels

Endpoint	LOAEC mean
Effect level	5.41 mg/m³ air (analytical)
Sex	male
Basis for effect level / Remarks	
Endpoint	NOEC mean
Effect level	1.39 mg/m³ air (analytical)
Sex	male
Basis for effect level / Remarks	Histopathology: based on the absence of substance-related effects,

in particular absence of a pulmonary response (inflammation reaction) (see details below "Details on results")

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

yes

Food consumption

no effects

Food efficiency

no effects

Water consumption

no data

Ophthalmoscopic examination

not examined

Haematology

not examined

Clinical chemistry

not examined

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

yes

Gross pathology

no effects

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY
no effects

BODY WEIGHT AND WEIGHT GAIN
Slight significant body-weight loss during the exposure period of 5 days in all dose groups. Normal bw gain thereafter.

ORGAN WEIGHTS

LUNG WEIGHT and LYMPH NODES:
Significant mean increases in relative and absolute lung weights of the mid- and high-dose groups (Tab. 11.1). No increases in weights of the tracheobronchial lymph nodes.

HISTOPATHOLOGY: NON-NEOPLASTIC
Histologically manifested changes were
- very slight hypertrophy of the brochiolar epithelium in 3/5 animals (mid dose) and slight hypertrophy in 4/5 (high dose). No case occurred in the recovery group.
- accumulation of alveolar macrophages accompanied by a few granulocytes/neutrophils in 3/5 animals (mid-dose) and 5/5 (high dose). In 3/5 high-dose animals, alveolar accumulation of macrophages was accompanied by infiltration of polymorphonuclear leukocytes (Tab. 10.1). Following recovery of 1 month, very slight macrophage accumulation was still present in the lungs 3/5 high-dose animals, but without epithelial changes and leukocyte infiltration. At that time lymph nodes also contained aggregates of macrophages [1/5 mid-dose, 5/5 high-dose] (Tab. 10.2).Following recovery of 3 months, focal accumulation of macrophages was still present in the lungs of 2/5 high-dose animals. The lymph nodes of 1/5 mid-dose and 5/5 high-dose animal still contained macrophage aggregates.

HISTORICAL CONTROL DATA (if applicable): no data

OTHER FINDINGS

CELL DIFFERENTIATION IN LAVAGE:
Dose-related stimulation of neutrophil granulocytes: After 5 d, the absolute and relative number of neutrophils increased significantly in both the mid- and high-dose group ($p<0.01$), the relative (not the absolute) number of macrophages decreased concomittantly (p. 21/22, Tab. 5.1 + 6.1). After 1-month recovery, the cell stimulating effect passed away, there were still slight, but statistically significant increases in the percentages of the neutrophil counts ($p<0.01$) with concomittant decreases in relative macrophage counts (Tab. 6.2), but no longer after 3 months (Tab. 6.3). No treatment-related changes were seen in the low-dose group.

BIOCHEMICAL PARAMETERS:
Significant increases in enzymes, protein, and the TNF-alpha levels were found at the mid- and high-dose exposure (mainly $p<0.01$; occasionally <0.05), which completely reversed after recovery (Tab. 7). The OH-proline content revealed no treatment-related changes.

SILICON CONTENT:
One day after exposure, 43 µg Si (average) were analysed in lungs of high-dose animals, which was

below detection limit after 1 month recovery (<15 µg). [note: no determinations carried out in the low and mid-dose groups]
No increased Si levels were observed in the lymph nodes (below detection limit (<15 µg).

Overall remarks, attachments

Overall remarks

The mid and high exposure concentration (analytical 5.41 and 25.3 mg/m^3) induced substance and dose-related effects which reflect an inflammatory response of the lung tissue. These tend to disappear during recovery, but slowly and not completely during the observation time. After 3 months recovery, macrophage accumulation was still present without tissue lesions. The lymph nodes were also affected.
No effects were noted at the low-concentration level (analytical 1.39 mg/m^3), except a transient body-weight loss noted throughout all treated groups. It is concluded that the NOEC (sub-acute) is at 1.39 mg/m^3. Based on a hypertrophic effect already observed at the mid-dose level, the LOAEC is defined as 5.41 mg/m^3. All synthetic amorphous silica was completely cleared from the lung, but clearance is different for various silica (see also other entries): for test substance very quickly. The granuloma-like lesions were not progressive, i.e. no silicogenic nodules formed (no silicosis). Survival was not affected in any of the groups. The only clinical sign noted with test substance was increased respiration rate. At the 1.3 mg-level, the effects were mild, completely cured after 13 wks recovery. There were no histologically manifested tissue changes. Therefore, this exposure concentration is considered the NOAEC for subchronic exposure, but depending on the pathological relevance placed on the observed effects, this NOAEC may be applicable to chronic exposure.

7.6 Genetic toxicity

7.6.1 Genetic toxicity in vitro

Endpoint study record: Genetic toxicity in vitro.001 Ames

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	20 Nov. - 12 Dec. 1989
Reliability	1 (reliable without restriction)		
Rationale for reliability	GLP guideline study		

Data source

Reference

Reference type	study report		
Author	San RHC, Springfield KA	**Year**	1989
Title	Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test)		
Bibliographic source	Unpublished report		
Testing laboratory	Microbiological Associates, Inc., USA	**Report no.**	T9085.501
Owner company	Cabot Corporation		
Company study no.		**Report date**	1989-12-28

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

gene mutation

Type of study

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

Test guideline

Qualifier according to

Guideline OECD Guideline 471 (Bacterial Reverse Mutation Assay)

Deviations

Principles of method if other than guideline

Plate incorporation assay

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Identifier other:

Identity The test substance is equivalent to NM 203.

Details on test material

CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5
- Analytical purity: >99%
- Lot/batch No.: 1H049
- Stability under test conditions: stable

Confidential details on test material

The test substance is equivalent to NM 203.

Method

Species/strain

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	with and without
Metabolic activation system	Aroclor induced rat liver S9 (adult male SD rats)
Species/strain	S. typhimurium TA 1538
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	with and without
Metabolic activation system	Aroclor induced rat liver S9 (adult male SD rats)

Test concentrations

667, 1000, 3333, 6667, and 10000 µg/plate

Vehicle

- Vehicle(s)/solvent(s) used: DMSO
- Justification for choice of solvent/vehicle: no data

Details on test system and conditions

Ames test
METHOD OF APPLICATION: in medium; in agar (plate incorporation)

DURATION- Exposure duration: no data

NUMBER OF REPLICATIONS: 3/concentration

NUMBER OF CELLS EVALUATED: seeded cells 10^8/plate

DETERMINATION OF CYTOTOXICITY
- Background lawn evaluated in pre-tests using TA 100

OTHER EXAMINATIONS:
- Other: Reversion rate

Evaluation criteria

no data

Statistics

Arithmetic averages with standard deviations

Results and discussions

Test results

Species/strain

Metabolic activation	with and without
Test system	other: Salmonella typhimurium TA98, TA100, TA1535, TA1537,TA1538
Genotoxicity	negative
Cytotoxicity	other: none
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Genetic toxicity in vitro.002 Gene mut

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	Nov./Dec. 1989
Reliability	1 (reliable without restriction)		
Rationale for reliability	Comparable to guideline study, well documented.		

Data source

Reference

Reference type	study report		
Author	Sigler CI, Harbell, JW	**Year**	1990
Title	CHO/HGPRT mutation assay		
Bibliographic source	Unpublished report		
Testing laboratory	Microbiological Associates, Inc., USA	**Report no.**	T9085.332
Owner company	Cabot Corporation		
Company study no.		**Report date**	1990-01-31

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

gene mutation

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier according to

Guideline OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)

Deviations

Principles of method if other than guideline

HPRT assay testing for reversion to resistance to the purine-analogue, 6-thioguanine, as result of a mutation in the X-chromosome-linked hypoxanthine-guanine phosphoribosyl transferase (HPRT).

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Identifier other:

Identity The test substance is equivalent to NM 203.

Details on test material

CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5
- Substance type: inorganic
- Physical state: solid
- Analytical purity: >99%- Lot/batch No.: 1H049
- Stability under test conditions: stable

Confidential details on test material

The test substance is equivalent to NM 203.

Method

Species/strain

Species/strain	Chinese hamster Ovary (CHO)
Details on mammalian cell lines (if applicable)	- Type and identity of media: Ham´s F-12 medium without hypoxanthine with 5 % FBS, 1 % penicilin-streptomycin and 1 % L-glutamine - Properly maintained: yes - Periodically checked for Mycoplasma contamination: yes - Periodically checked for karyotype stability: no data - Periodically "cleansed" against high spontaneous background: no data -Source: A. Hsie, Oak Ridge National Laboratories, directly received in frozen state
Additional strain characteristics	
Metabolic activation	with and without
Metabolic activation system	Aroclor induced rat liver S9 (adult male SD rats)

Test concentrations

10, 50, 100, 150, and 250 µg/ml (without S9) and 100, 200, 300, 400, and 500 µg/ml (with S9)

Vehicle

- Vehicle(s)/solvent(s) used: DMSO, 1 % final concentration

Controls

Negative controls	yes untreated
Solvent / vehicle controls	yes DMSO
True negative controls	
Positive controls	yes
Positive control substance	other: Ethyl methanesulphonate (without S9) and benzo(a)pyrene (with S9)

Remarks

Details on test system and conditions

METHOD OF APPLICATION: in medium

DURATION

- Preincubation period: 18 - 24 hours at 37 ± 1 °C, 5 x10^5 cell/25 ml flask in 5 ± 1% CO_2 atmosphere
- Exposure duration: 5 h (without and with S9)
- Expression time (cells in growth medium): Post exposure: 18 - 24 h, followed by 7 - 9 days including subculturing of 2 - 3 day intervals
- Selection time (if incubation with a selection agent): In the presence of 6-thioguanine, 7 days
- Fixation time (start of exposure up to fixation or harvest of cells): 15 - 17 days

SPINDLE INHIBITOR (cytogenetic assays): --

STAIN (for cytogenetic assays):
10 % Giemsa

NUMBER OF REPLICATIONS: 2

NUMBER OF CELLS EVALUATED:
2 x10^5 cells /100 mm dish (five-fold) = in total 10^6 cells per concentration

DETERMINATION OF CYTOTOXICITY
- in parallel as cloning efficiency (triplicates with each 100 cells/60 mm dish)

Evaluation criteria

A response was not considered positive unless the mutant frequency exceeded 20 mutants per 10^6 clonable cells. Significant if twice that of background and at least 11 mutants per 10^6 cells above background (solvent and untreated control). Positive if dose-dependen increase in mutant frequency combined with significant increase at one or more test concentrations.Suspect if no dose-response.

Statistics

Frequency of spontaneous mutations (if showing wide variation): C.I. (Conf. Interval) calculated by application of the one-sided student´s test from the historical background rate (upper limit of C.I.: 11 spontaneous mutants/10^6 cells).

Results and discussions

Test results

Species/strain

Metabolic activation	with and without
Test system	strain/cell type: Chinese hamster ovary (CHO) cells
Genotoxicity	negative see under "Remarks on results"
Cytotoxicity	other: Not pronounced in the mutation test, while a concurrent cytotoxicity test showed considerable inhibition of the cloning efficiency over the same dose ranges.
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS:

none

RANGE-FINDING/SCREENING STUDIES:
9 concentrations between 0.1 and 500 µg/ml tested with and without S9, toxicity based on colony-forming efficiency

COMPARISON WITH HISTORICAL CONTROL DATA:
not presented

Remarks on results including tables and figures

From Report Tab. 3 and 4
With S9 (all data relating to x mutants/10^6 cells)
negative controls: 1 and 7.2
positive control: 351
treated cells: no dose response
1 and <1 at higher dose levels (100 to 250 µg/ml) with cytotoxicity noted at 250 µg/ml.
7.3 and 10.3 at the lower dose levels (10 and 50 µg/ml, respectively).
Without S9 (all data relating to x mutants/10^6 cells)
negative controls: 0.8 and 8.0
positive control: 184
treated cells: no dose response
<1 to 8.2
no pronounced cytotoxicity noted at any dose level

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Genetic toxicity in vitro.003 ChromAb_CHO

Administrative Data

Purpose flag	key study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	Dec. 1989
Reliability	1 (reliable without restriction)		
Rationale for reliability	Comparable to guideline study, well documented.		

Data source

Reference

Reference type	study report		
Author	Putman DL, Morris, MJ	**Year**	1990
Title	Chromosome aberrations in Chinese hamster (CHO) cells		
Bibliographic source	Unpublished report		
Testing laboratory	Microbiological Associates, Inc., USA	**Report no.**	T9085.337
Owner company	Cabot Corporation		
Company study no.		**Report date**	1990-02-20

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier according to

Guideline OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)

Deviations yes 100 instead of 200 metaphases were scored.

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Identifier other:

Identity The test substance is equivalent to NM 203.

Details on test material

CAS-Name: Silica, amorphous, fumed, cryst.-free;
CAS-No.: 112945-52-5
- Substance type: inorganic
- Physical state: solid
- Analytical purity: >99%
- Lot/batch No.: 1H049
- Stability under test conditions: stable

Confidential details on test material

The test substance is equivalent to NM 203.

Method

Species/strain

Species/strain	Chinese hamster Ovary (CHO)
Details on mammalian cell lines (if applicable)	- Type and identity of media: Ham´s F-12 medium without hypoxanthine with 5 % FBS, 1 % penicilin-streptomycin and 1 % L-glutamine - Properly maintained: yes - Periodically checked for Mycoplasma contamination: yes - Periodically checked for karyotype stability: no data - Periodically "cleansed" against high spontaneous background: no data -Source: A. Hsie, Oak Ridge National Laboratories, directly received in frozen state
Additional strain characteristics	
Metabolic activation	with and without
Metabolic activation system	Aroclor induced rat liver S9 (adult male SD rats)

Test concentrations

38, 75, 150, 300 µg/ml (without S9) and 250, 500, 750, 1000 µg/ml (with S9)

Vehicle

- Vehicle(s)/solvent(s) used: DMSO
- Justification for choice of solvent/vehicle: No data

Controls

Negative controls	yes untreated cells
Solvent / vehicle controls	yes DMSO
True negative controls	
Positive controls	yes
Positive control substance	other: Triethyleneamine without S9, cyclophosphamide with S9
Remarks	

Details on test system and conditions

METHOD OF APPLICATION:

in medium; preincubation

DURATION
- Preincubation period: 16 - 24 hours at 37 ± 1 °C (5 x10^5 cell/25 ml flask in 5 ± 1% CO_2 atmosphere
- Exposure duration: 18 h (without S9); 2 h (with S9)
- Expression time (cells in growth medium): 11 h (for S9-activated cultures)
- Selection time (if incubation with a selection agent): addition of Colcemid (0.1 µg/ml) 2 h prior to sampling of metaphase cells
- Fixation time (start of exposure up to fixation or harvest of cells): Sum Exposure + expression time

SPINDLE INHIBITOR (cytogenetic assays):
Colcemid STAIN (for cytogenetic assays): 5 % Giemsa

NUMBER OF REPLICATIONS: 2

NUMBER OF CELLS EVALUATED: 100

DETERMINATION OF CYTOTOXICITY
- Method: mitotic index; cell cycle delay

OTHER EXAMINATIONS:
- Determination of polyploidy: no data
- Determination of endoreplication: no data
- Other: chromatid-type and chromosome-type aberrations (breaks, various exchange figures)
Chromatid and isochromatid gaps were recorded, but not included in the analysis.

OTHER:
Mitotic index was recorded as percentage of cells in mitosis per 500 cells counted.

Evaluation criteria

Dose-response with one or more concentrations elevated in relation to the solvent control ($p \leq 0.05$). A significant increase at the highest dose only with no dose-response was considered suspect. A significant increase at one dose level other than the high dose with no dose-response was considered equivocal.
Validity: The number of cells with chromosome aberrations in the negative and solvent control must be no greater than 6 %. The percentage of aberrations in the positve controls must be statistically increased ($p \leq 0.05$ Fisher´s exact test) relative to the untreated control.

Statistics

Fisher´s exact test for pairwise comparison of the aberrations in test and control cultures. The Cochran-Armitage test was applied for measure dose-responsiveness.

Results and discussions

Test results

Species/strain	Chinese hamster Ovary (CHO)
Metabolic activation	with and without
Test system	other: Chinese hamster ovary (CHO) cells
Genotoxicity	negative see Table "Remarks on results" below
Cytotoxicity	yes see "Additional information" below
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS
none mentioned

RANGE-FINDING/SCREENING STUDIES:
9 concentration between 0.1 - 1000 µg/mlwith and without S9

COMPARISON WITH HISTORICAL CONTROL DATA:
no data

ADDITIONAL INFORMATION ON CYTOTOXICITY:
Mitotic index (MIx) and cell cycle kinetics (M1) (from Report Tab. 1 and 2):

	-S9		+S9	
	MIx[%]	M1[%]	MIx[%]	M1[%]
DMSO control	5	2	3.4	3
0.1 µg/L	3.8	2	2.6	3
10 µg/L	3.2	7	3.2	10
30 µg/L	2.6	17	2.8	7
100 µg/L	2.2	14	3.2	3
300 µg/L	1.2	27	1.0	24

Cell proliferation began to be inhibited at 30 µg/L (-S9) and 300 µg/L (+S9). Simulataneously, the cell cycle became retarded with an accumulation of cells in the M1 phase. Neither in the control nor in the treated cultures, cells were observed in the M3 phase (except 1 instance in the DMSO control).

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Genetic toxicity in vitro.004 UDS

Administrative Data

Purpose flag supporting study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 28 Nov. - 22 Dec. 1989

Reliability 1 (reliable without restriction)

Rationale for reliability GLP guideline study

Data source

Reference

Reference type	study report		
Author	Curren, RD	**Year**	1989
Title	Unscheduled DNA synthesis in rat primary hepatocytes		
Bibliographic source	Unpublished report		
Testing laboratory	Microbiological Associates, Inc., USA	**Report no.**	T9085.380
Owner company	Cabot Corporation		
Company study no.		**Report date**	1989-12-28

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro

Test guideline

Qualifier according to

Guideline OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)

Deviations

Principles of method if other than guideline

William, G. M.: Chemical Mutagens, 4, 61-79, 1979

GLP compliance

yes

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Identifier other:

Identity The test substance is equivalent to NM 203.

Details on test material

CAS-Name: Silica, amorphous, fumed, cryst.-free;
CAS-No.: 112945-52-5
- Substance type: inorganic
- Physical state: solid
- Analytical purity: >99%
- Lot/batch No.: 1H049
- Stability under test conditions: stable

Confidential details on test material

The test substance is equivalent to NM 203.

Method

Species/strain

Species/strain hepatocytes: primary culture, rat

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation without

Metabolic activation system

Test concentrations

10, 30, 100, 300, and 1000 µg/ml (5 concentrations tested)

Vehicle

- Vehicle(s)/solvent(s) used: DMSO, 1 % final concentration

Controls

Negative controls yes untreated media control

Solvent / vehicle controls yes DMSO

True negative controls

Positive controls yes

Positive control substance 7,12-dimethylbenzanthracene

Remarks

Details on test system and conditions

NUMBER OF REPLICATIONS: 3

INCUBATION TIME:
18 to 20 h in the presence of silica

NUMBER OF CELLS EVALUATED:
150 (3 x50)

DETERMINATION OF CYTOTOXICITY
- LDH release

Evaluation criteria

Validity: Positive control with significant increases in net nuclear grain count, and negative control with less than 15 % of the cells in repair stateand solvent control with net nuclear grain count of less than 1.
Statistical significance: increase in mean nuclear count by at least 5 counts over control ==> significant for the specific dose level.
Positive: dose-related increase and at least one dose with significant increase or positive: significant increases at two successive doses in the absence of a dose response.
Equivocal: significant increase at one doses in the absence of a dose response.

Results and discussions

Test results

Species/strain

Metabolic activation	without
Test system	other: Primary rat hepatocytes
Genotoxicity	negative
Cytotoxicity	other: 260 - 500 µg/ml: rel. toxicity approx. 50 %
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Remarks on results including tables and figures

No dose response was observed. Average net grain counts per nucleus were <1 in the negative controls and ranged from <1 to 2.1 in the treated cultures. Average net grain counts per nucleus were 30 - 32 in the positive control. Average net grain counts per nucleus ranged from 1 to 2.1 in the treated cultures.

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Genetic toxicity in vitro_NM 203_COMET 16-HBE

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	UAB (SP)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in mammalian cells for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human bronchial epithelial 16-HBE cells

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

5/10/20/ 40/60 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h
Use of FpG to detect oxidative damage

Evaluation criteria

Median percentage of DNA in the tail (% Tail DNA) with > 200 cells scored per dose

Statistics

one way ANOVA

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_COMET 16 HBE_UAB.docx / 24.08 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

SAS NM-203 induces neither DNA strand breaks nor DNA oxidative damage in 16-HBE cells at both 3 h and 24 h at the tested dose with the alkaline comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_COMET A549

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type study report

Author H Norppa **Year** 2013

Title Deliverable 5: In vitro testing strategy for nanomaterials including database

Bibliographic source

Testing laboratory IPH (B) **Report no.**

Owner company

Company study no. **Report date**

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in mammalian cells for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human alveolar epithelial A549 cells

Details on mammalian cell lines (if

applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

2.56/25.6/256 /512 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 hUse of FgG to detect oxidative damage

Evaluation criteria

Median percentage of DNA in the tail (% Tail DNA) with >200 cells scored per dose

Statistics

Kruskall wallis one-way analysis

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_COMET A549_IPH.docx / 23.94 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other:
Without FpG: Negative at 3h ,
Positive at 24h: increase in the % Tail DNA at 2 doses (25.6 and 256 µg/ml).
With FpG: Negative at 3h,
Positive at 24h: increase in the % Tail DNA at 2 doses (25.6 and 256 µg/ml)

Conclusions

SAS NM-203 induces DNA strand breaks in A 549 cells at 24 h but not 3 h at the tested dose with the alkaline comet assay.
SAS NM-203 induces oxidative DNA at 24 h but not 3 h with the FpG-modified comet assay

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_COMET BEAS-2B

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	IPH (B)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in mammalian cells for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human bronchial epithelial BEAS 2B cells

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

2.56/25.6/ 256/512 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h Use of FpG to detect oxidative damage

Evaluation criteria

Median percentage of DNA in the tail (% Tail DNA) with >200 cells scored per dose

Statistics

Kruskall wallis one-way

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_COMET BEAS-2B_IPH.docx / 22.33 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

positive
Without FpG: Positive at 3 h: increase in the % Tail DNA at 3 dose (2.56, 25.6 and 256 µg/ml).
With FpG: Positive: increase in the % Tail DNA at 3 doses (2.56, 256 and 512 µg/ml)

Conclusions

SAS NM-203 induces DNA strand breaks in BEAS-2B cells at 3 h with the alkaline comet assay. SAS NM-203 induces oxidative DNA damage following 3 h treatment at 3 doses with the FpG-modified alkaline comet assay

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_COMET BEAS-2B_Robin Test

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	IPH (B), FIOH (FL), NIOM (PL), IMB-BAS (BG), UAB (SP), INSA (PT)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in mammalian cells for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human bronchial epithelial BEAS 2B cells

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

8/32/64 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 24 h

Evaluation criteria

Median percentage of DNA in the tail (% Tail DNA) with 200 cells scored per dose

Statistics

ANOVA

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_COMET BEAS-2B_Robin Test.docx / 21.88 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other:
Positive: dose-dependant increase in the % Tail DNA in 3 out 6 labs.
Negative: no increase in the % Tail DNA in 3 out 6 labs

Conclusions

SAS NM 203 induces DNA strand breaks at 24 h in BEAS-2B with the alkaline comet assay in 3 out 6 labs whereas negative results are observed in the other labs

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_COMET Caco-2

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	Anses (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in mammalian cells for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain	mammalian cell line, other: Undifferentiated human intestinal Caco-2 cell line
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

2.56/25.6/256 /512 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h
Use of FpG to detect oxidative DNA damage

Evaluation criteria

Median percentage of DNA in the tail (% Tail DNA) with >200 cells scored per dose

Statistics

Kruskall wallis one-way

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_COMET Caco2_IPH.docx / 24.37 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other:
Without FpG:
Positive at 3h and 24 h: increase in the % Tail DNA at 2 doses.
With FpG:
Positive at 3h: increase in the % Tail DNA at 2 doses , Equivocal at 24 h (increase in the % Tail DNA at one dose).

Conclusions

SAS NM-203 induces DNA strand breaks in A 549 cells at both 3 h and 24 h with the alkaline comet assay. SAS NM-203 induces oxidative DNA damage at 3 h and an equivocal response at 24 h with the FpG-modified comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_COMET Caco-2_Robin Test

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	Anses (F), NRCWE (DK), BfR (GER), IPL (F), INRS (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in mammalian cells for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: Undifferentiated human intestinal Caco-2 cell line

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

64/128/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h

Evaluation criteria

Median percentage of DNA in the tail (% Tail DNA) with 200 cells scored per dose

Statistics

ANOVA

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_COMET Caco2_Robin Test.docx / 21.27 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other: Negative in 3 out 5 labs: no increase in the % Tail DNA . Positive in 2 out 5 labs: increase in the % Tail DNA

Conclusions

SAS NM 203 induces DNA strand breaks at 24 h in Caco-2 cells with the alkaline comet assay in 2 out 5 experiments whereas negative response was observed in 3 out 5 experiments

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MLA TK

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	IPL (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

gene mutation

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier according to

Guideline OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test) MLA TK

Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: L5178Y TK ±mouse lymphoma cells

Details on mammalian cell lines (if

applicable)

Additional strain characteristics

Metabolic activation without

Metabolic activation system

Test concentrations

32/64/128/256/625/1250/2500/5000 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time 24 h

Evaluation criteria

Mutation frequency forSmall colonies + Large colonies (x106 cells)

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_MLA TK_IPL.docx / 20.71 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

SAS NM 203 is not mutagenic in L5178Y TK ±mouse lymphoma cells at the tested doses
With the in vitro mammalian cell gene mutation test.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MN 16-HBE

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	IPL (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)

Deviations no Test without cytochalasin B

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human bronchial 16-HBE cell line

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations
8/12/ and 16 µg/ml

Vehicle
BSA 0.05 % prepared in milliQ water

Evaluation criteria
1000 cells scored per culture; 2000 cells scored per condition

Statistics
Chi square or Fisher

Overall remarks, attachments

Attached full study report
NGTX_gentox_invitro_NM203_MN 16HBE_IPL.docx / 21.51 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results
negative

Conclusions
SAS NM-203 does not induce aneugenic/clastogenic damage in 16-HBE cells at the tested dose following a 41h incubation with the cytokinesis-block micronucleus assay.

Cross-reference to other study
http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MN A549

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	INRS (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)

Deviations yes Cytochalasin B added 6 h after NM

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human alveolar epithelial A549 cells

Details on mammalian cell lines (if

applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

32/64/128/256/512 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

2 independant experiments

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square or Fisher

Applicant's summary and conclusion

Interpretation of results

other:
Negative in the 1st experiment: no increase in the frequency of binucleated cells with micronuclei, Equivocal in the 2nd experiment: increase in the frequency of binucleated cells with micronuclei at one dose (128 µg/ml)

Conclusions

SAS NM-203 does not induce aneugenic/clastogenic damage in A 549 at the tested dose in 1 out 2 experiment.
Equivocal response was induced in the 2nd experiment with the cytokinesis-block micronucleus assay.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MN BEAS-2B

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	FIOH (FL)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)

Deviations yes Ctochalasin b added 6 h after NM

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human bronchial BEAS-2B cell line

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations
4/8/16/32/64 µg/ml

Vehicle
BSA 0.05 % prepared in milliQ water

Evaluation criteria
1000 cells scored per culture; 2000 cells scored per condition

Statistics
Chi square or Fisher

Overall remarks, attachments

Attached full study report
NGTX_gentox_invitro_NM203_MN BEAS-2B_FIOH.docx / 21.1 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results
ambiguous increase in the frequency of binucleated cells with micronuclei at one dose (8 µg/ml)

Conclusions
SAS NM-203 induces equivocal aneugenic/clastogenic in BEAS-2B with damage at one dose following a 48h incubation with the cytokinesis-block micronucleus assay.

Cross-reference to other study
http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MN BEAS-2B_Robin Test

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	FIOH (FL), NIOM (PL), PH (B), IMB-BAS (BG), Insa (PT)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)

Deviations yes Ctochalasin b added 6 h after NM

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: human bronchial BEAS-2B cell line

Details on mammalian cell lines (if

applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

8/32/64 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square or Fisher

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_MN BEAS-2B_Robin test.docx / 21.91 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other: Negative in 3 out 6 labs,
Equivocal in 1 lab: increase in the frequency of binucleated cells with micronuclei at one dose,
Positive in 2 labs: increase in the frequency of binucleated cells with micronuclei

Conclusions

SAS NM-203 induces equivocal aneugenic/clastogenic in BEAS-2B with damage at one dose following a 48 h incubation with the cytokinesis-block micronucleus assay.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MN Caco-2

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	Anses (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)

Deviations yes Cytochalasin B added 24 h after NM

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: Undifferentiated human cell line Caco-2

Details on mammalian cell lines (if

applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

9.5/28/85/128/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

culture medium used: MEM medium supplemented with 5 % Fetal Calf Serum3 independant experiments

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square (one tailed)

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_MN Caco2_Anses.docx / 21.49 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

other: Statistical increase in the frequency of binucleated cells with micronuclei in 2 out 3 experiments. Dose-response relationship was observed in one experiment only (exp 3).

Conclusions

In 2 out 3 experiments, an induction of chromosomal damage is observed in Caco-2 cells with the cytokinesis-block micronucleus assay. SAS NM-203 is genotoxic at the highest doses in vitro.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MN Caco-2_Robin Test

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	Anses (F), BfR (GER), IPL (F), NRCWE (DK), INRS (F), RIVM (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)

Deviations yes Cytochalasin B added 24 h after NM

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain mammalian cell line, other: Undifferentiated human cell line Caco-2

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations
9.5/28/85/128/256 µg/ml

Vehicle
BSA 0.05 % prepared in milliQ water

Details on test system and conditions
culture medium used: MEM medium supplemented with 5 % Fetal Calf Serum3 independant experiments

Evaluation criteria
1000 cells scored per culture; 2000 cells scored per condition

Statistics
Chi square (one tailed)

Overall remarks, attachments

Attached full study report
NGTX_gentox_invitro_NM203_MN Caco-2_Robin test.docx / 22.26 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results
other: Positive in 3 labs: dose-dependant increase in the frequency of binucleated cells with micronuclei, Negative in 3 labs

Conclusions
SAS NM 203 induces aneugenic/clastogenic response with dose-dependant effect in 3 out 6 labs in Caco-2 cells cells. Negative response was observed in the 3 other labs with the Cytokinesis-block micronucleus assay.

Cross-reference to other study
http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vitro_NM 203_MN Lymphocytes

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	IPL (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but not willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)

Deviations yes Cytochalasin b added 6 h after NM

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Method

Species/strain

Species/strain primary culture, other: human primary peripheral blood lymphocytes

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation

Metabolic activation system

Test concentrations

256/312.5/625/1250 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square or Fisher

Overall remarks, attachments

Attached full study report

NGTX_gentox_invitro_NM203_MNLympho_IPL.docx / 22.55 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

SAS NM-203 does not induce aneugenic/clastogenic damage in primary human blood lymphocytes cells at the tested dose following a 48 h incubation.

Cross-reference to other study

http://www.nanogenotox.eu/

7.6.2 Genetic toxicity in vivo

Endpoint study record: Genetic toxicity in vivo.001

Administrative Data

Purpose flag key study (X) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability Comparable to guideline study, containing scientificallyjustified modifications, no validated standard test in vivo (see: Method).

Data source

Reference

Reference type	publication		
Author	Johnston CJ, Driscoll KE, Finkelstein JN et al.	**Year**	2000
Title	Pulmonary chemokine and mutagenic responses in rats after subchronic inhalation of amorphous and crystalline silica		
Bibliographic source	Toxicol. Sci. 56, 405-413		
Testing laboratory	University Rochester, NY	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

data published

Cross-reference to same study

see also Chapter 7.5 (Repeated dose)

Materials and methods

Type of genotoxicity

gene mutation

Type of study

other: mammalian cell gene mutation assay: ex-vivo/in-vitro HPRT assay

Test guideline

Qualifier no guideline available

Guideline

Deviations

Principles of method if other than guideline

Method: ex-vivo/in-vitro HPRT assay, no standard guideline available, HPRT part likely based on OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test) as well as previous inhalation on OECD

Guideline 413 (Subchronic Inhalation Toxicity: 90-Day, but limited performance, only males, one high exposure level, without comprehensive organtoxicology and pathology as well as without clinical chemistry/haematology etc.

GLP compliance

no data

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

- CAS-Name: Silica, amorphous, fumed, cryst.-free; CAS-No.: 112945-52-5 (note: specified as "precipitated" in the report, apparently erroneous).
- Substance type: inorganic
- Physical state: solid

Test animals

Species

rat

Strain

Fischer 344

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Age at study initiation: no data
- Weight at study initiation: 200 - 250 g

no further data

Administration / exposure

Route of administration

inhalation

Details on exposure

TYPE OF INHALATION EXPOSURE:
whole body
GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: 300-L horizontal laminar-flow chamber, compartmentalised

- System of generating particulates/aerosols: srew-feed mechnaism (ACCURate, Whitewater) in combination with a Venturi-type dust feeder
- Temperature, humidity, pressure in air chamber: no data
- Air flow rate: no data- Air change rate: no data
- Method of particle size determination: no data

TEST ATMOSPHERE
- Brief description of analytical method used: no data
- Samples taken from breathing zone: no data

Duration of treatment / exposure

13 wks

Frequency of treatment

6 h/d, 5 d/wk

Post exposure period

no, not for this endpoint of the study

Doses / concentrations

50 mg/m^3

Basis nominal conc.

50.4 ± 19 mg/m^3

Basis analytical conc.

No. of animals per sex per dose

no data, only males, probably 4 animals per endpoint

Control animals

yes, concurrent no treatment

Positive control(s)

Crystalline silica was examined simulataneously.

Examinations

Tissues and cell types examined

The testing programme included cellular and biochemical
Bronchoalveolar Lavage Fluid Analysis (BLA) on inflammatory markers, histopathology, inflammatory cytokine gene expression, immunohistochemisty for DNA damage, and mutagenesis in alveolar epithelial cells.

Evaluation criteria

not specified

Statistics

Analysis of variance, Dunnett´s test for determination of differences between control and treated groups.

Any other information on materials and methods incl. tables

GENERAL
Comparative study including synthetic amorphous and crystalline silica:

Rationale for exposure: Elucidation of mutagenic events in the lung in response to fibrogenic and tumour-inducing crystalline silica and non-fibrogenic amorphous silica particles. Thereby, characterisation of pulmonary inflammation, cytotoxicity, biopersistence, and mutagenicity of subchronic inhalation in rats.
Dose selection: high inflammatory but no lethal dose. The testing programme included cellular and biochemical
Bronchoalveolar Lavage Fluid Analysis (BAL) on inflammatory markers, histopathology, inflammatory cytokine gene expression (MIP-2), immunohistochemistry for DNA damage (terminal transferase dUTP nick-end-labeling = TUNEL staining), and mutagenesis in alveolar epithelial cells.
HPRT ASSAY For mutagenesis,alveolar type-II cells were isolated from BAL after 13 wks of dust exposure and subjected to the HPRT gene-mutation assay.Freshly isolated alveolar type-II cells were seeded at 2 x10^5 epithelial cells/flask (total 6 T25 flasks), incubation overnight at 37 °C and 5% CO_2. The cells were washed and the remainder cell layer fed with medium containing6-thioguanine (selective mdium). The cells were cultured for 14 - 21 days in selective medium (6-thioguanine containing) re-fed every other day prior to fixation.
Mutant frequency: Number of colonies/treatment divided by the plating efficiency/10^6 cells = mutants/10^6 cells.

Results and discussions

Test results

Sex	male
Genotoxicity	negative Alveolar type-II cells isolated from the 50-mg/m3 rat group showed no increased mutation frequency as compared to the control.
Toxicity	no effects Viability in lung-cell isolates was not impaired (see Report p. 409).
Vehicle controls valid	not applicable
Negative controls valid	yes
Positive controls valid	yes

Remarks on results including tables and figures

No differences were detected between treatment groups in the yield of alveolar type-II cells or the viability of lung-cell isolates. There was no increase in 6TG-resistant mutants (av. approx. 4 mutants/10^6 cells vs. control (7.6 ± 3.4 mutants/10^6 cells in the air control) immediately after exposure of rats to 50 mg/m^3 of amorphous silica, whereas after exposure to crystalline silica (3 mg/m^3), the mutant frequency was significantly enhanced (approx. 33 mutants/10^6 cells) despite the more than 10-fold lower exposure concentration (Report Fig. 4).

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Genetic toxicity in vivo_NM 203_COMET Gavage

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2012
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	Anses (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM 203_MN Bone marrow Gavage and NM 203_MN Colon

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in rodents for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Test animals

Species
rat

Strain
Sprague-Dawley

Sex
male

Administration / exposure

Route of administration
oral: gavage

Vehicle(s)
Normal saline buffer (NaCl 0.90% w/v)

Duration of treatment / exposure
3 administrations: 1st at 0, 2nd at 24 and 3rd 45 h
Sampling 3 h after the last administration

Doses / concentrations
5, 10, 20 mg/kg bw/d

Basis nominal conc.

No. of animals per sex per dose
5

Control animals
yes

Positive control(s)
Methyl MethaneSulfonate (80 mg/kg for 2 first admin-100 mg/kg for 3rd admin)

Examinations
Tissues and cell types examined
blood, bone marrow,liver, kidney,spleen, colon, duodenum

Details of tissue and slide preparation
Use of FpG to detect oxidative DNA damage

Evaluation criteria
Median % Tail DNA of 100 nucleoids

Statistics
one-way ANOVA and t test for negative vs positive controls

Overall remarks, attachments

Attached full study report

NGTX_gentox_invivo_NM203_COMET Gavage_Anses.docx / 21.08 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

negative No DNA damage was observed following gavage with SAS NM-203 irrespective of the NM and the organ investigated.
Using FpG, no oxidative DNA damage was induced in the MN treated animals compared to the control.

Conclusions

SAS NM-203 is not genotoxic in rats at the tested concentrations following a short-term exposition via oral route.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vivo_NM 203_COMET Instillation

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2013
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	INRS (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM 203_MN Bone marrow Instillation

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

single cell gel/comet assay in rodents for detection of DNA damage

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Administration / exposure

Route of administration

intratracheal

Vehicle(s)

Normal saline buffer (NaCl 0.90% w/v)

Duration of treatment / exposure

1 administration at 0, 24 and 45 h
Sampling: 3 h after the last administration

Doses / concentrations

3, 6, 12 mg/kg bw/d

Basis nominal conc.

No. of animals per sex per dose

5

Control animals

yes

Positive control(s)

Methyl MethaneSulfonate (50mg/kg for first admin-25 mg/kg for the 2nd and 3rd admin) and N-ethyl-N-nitrosurea (ENU) by gavage (25 mg./kg)

Examinations

Tissues and cell types examined

BAL cells, lung,blood,liver, spleen, kidney, bone marrow

Details of tissue and slide preparation

Use of FpG to detect oxidative DNA damage

Evaluation criteria

Median % Tail DNA of 100 nucleoids

Statistics

one-way ANOVA and t test for negative vs positive controls

Overall remarks, attachments

Attached full study report

NGTX_gentox_invivo_NM203_COMET instillation_Inrs.docx / 21.13 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

SAS NM-203 is not genotoxic in rats at the tested concentrations following a short-term exposition via intratracheal instillation

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vivo_NM 203_MN Bone marrow Gavage

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2013
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	Anses (F); Insa(PT) for scoring	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM 203_COMET gavage and NM 203_MN Colon Gavage

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier according to

Guideline OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

Normal saline buffer (NaCl 0.90% w/v)

Duration of treatment / exposure

3 administrations: 1st at 0, 2nd at 24 and 3rd at 45 h
Sampling: 3 h after the last administration

Doses / concentrations

5, 10, 20 mg/kg bw/d

Basis nominal conc.

No. of animals per sex per dose

5

Control animals

yes

Positive control(s)

Methyl Methane Sulfonate (80 mg:kg bw for 2 first admin-100 mg/kg bw for 3rd admin)

Examinations

Evaluation criteria

2000 immature erythrocytes per rat

Statistics

Chi-square test with Yate's correction

Overall remarks, attachments

Attached full study report

NGTX_gentox_invivo_NM203_MN Bone marrow Gavage_Anses.docx / 20.17 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

negative
No statistically significant decrease in the ratio PCE to NCE was observed in the NM-203 treated groups when compared to the negative control group. As a consequence, no proof of systemic exposure was evidenced. No clastogenicity/aneugenicity was observed

Conclusions

SAS NM-203 is not genotoxic in rats at the tested concentrations following a short-term exposure via oral route.

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vivo_NM 203_MN Bone marrow Instillation

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2012
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	INRS (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM 203_COMET Instillation

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier according to

Guideline OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Administration / exposure

Route of administration

intratracheal

Vehicle(s)

Normal saline buffer (NaCl 0.90% w/v)

Duration of treatment / exposure

3 administrations: 1st at 0, 2nd at 24h and the 3rd at 45 h
Sampling: 3 h after the last administration

Doses / concentrations

3, 6, 12 mg/kg

Basis nominal conc.

No. of animals per sex per dose

5

Control animals

yes

Positive control(s)

Methyl Methane Sulfonate (50mg/kg for first admin-25 mg/kg for the 2nd and 3rd admin) and N-ethyl-N-nitrosurea (ENU) by gavage (25 mg./kg)

Examinations

Tissues and cell types examined

Bone marrow

Details of tissue and slide preparation

Methyl MethaneSulfonate (50mg/kg for first admin-25 mg/kg for the 2nd and 3rd admin)

Evaluation criteria

2000 immature erythrocytes per rat

Statistics

Chi square

Overall remarks, attachments

Attached full study report

NGTX_gentox_invivo_NM203_MN Bone marrow_instillation_Inrs.docx / 41.94 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

negative No statistically significant decrease in the ratio PCE to NCE was observed in the NM-203 treated groups when compared to the negative control group. As a consequence, no proof of systemic exposure was evidenced. No clastogenicity/aneugenicity was observed

Conclusions

SAS NM-203 is not genotoxic in rats at the tested concentrations following a short-term exposition via intratracheal instillation

Cross-reference to other study

http://www.nanogenotox.eu/

Endpoint study record: Genetic toxicity in vivo_NM 203_MN Colon Gavage

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS

Study result type experimental result **Study period** 2012

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2013
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	Anses (F)	**Report no.**	
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but not willing to share

Cross-reference to same study

NM 203_COMET Gavage and NM 203_MN Bone marrow Gavage

Materials and methods

Type of genotoxicity
chromosome aberration

Type of study
other: Micronucleus assay in colon

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 203

Test animals

Species
rat

Strain
Sprague-Dawley

Sex
male

Administration / exposure

Route of administration
oral: gavage

Vehicle(s)
Normal saline buffer (NaCl 0.90% w/v)

Duration of treatment / exposure
3 administrations: 1st at 0, 2nd at 24 and 3rd at 45 h
Sampling 3 h after the last administration

Doses / concentrations
5, 10, 20 mg/kg bw/d

Basis nominal conc.

Control animals
yes

Positive control(s)
Methyl Methane Sulfonate (80-100 mg/kg)

Examinations

Tissues and cell types examined

Epithelial cell from the colon

Evaluation criteria

at least 1000 cells per rats

Statistics

Fisher exact test with Yate's correction

Overall remarks, attachments

Attached full study report

NGTX_gentox_invivo_NM203_MN colon Gavage_Anses.docx / 18.49 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

Applicant's summary and conclusion

Interpretation of results

Ambiguous
A significant increase in the frequency of micronucleated cells was observed in the rats treated with SAS NM-203 at the lowest dose only (5 mg/kg).

Conclusions

Induction of genotoxic effects were observed for the lowest dose only.
The genotoxicity of SAS NM-203 is equivocal following a short-term exposure via oral route.

Cross-reference to other study

http://www.nanogenotox.eu/

7.7 Carcinogenicity

7.8 Toxicity to reproduction

7.8.1 Toxicity to reproduction

Endpoint study record: Toxicity to reproduction.001

Administrative Data

Purpose flag	supporting study (X) robust study summary ( ) used for classification ( ) used for MSDS		
Study result type	experimental result	**Study period**	Start: 15 Oct. 1962
Reliability	2 (reliable with restrictions)		
Rationale for reliability	Significant methodological deficiences, acceptable as screening		

Data source

Reference

Reference type	study report		
Author	Leuschner F	**Year**	1963
Title	Ueber die chronische Toxizität		
Bibliographic source	Unpublished report		
Testing laboratory		**Report no.**	
Owner company	Evonik Degussa GmbH		
Company study no.	Degussa AG - US-IT-No. 63-0001-DKT	**Report date**	1963-01-02

Data access

other: data owner or letter of access

Data protection claimed

yes, but willing to share

Materials and methods

Test type

one-generation study

Test guideline

Qualifier equivalent or similar to

Guideline OECD Guideline 415 (One-Generation Reproduction Toxicity Study)

Deviations yes no complete one generation study according to current standards: too low number of animals and examinations, one dose only, dose selection unclear (relatively low dose selected).

Principles of method if other than guideline

Method: 5 instead of 20 females, 1 male per 5 females (mating ratio 1:5 instead of 1:2); mating over 14 d, only 1 male used per treated and control group

GLP compliance

no

Test materials

Test material equivalent to submission substance identity

yes

Test material identity

Identifier CAS number

Identity 7631-86-9

Identifier EC number

Identity 231-545-4

Identifier IUPAC name

Identity dioxosilane

Details on test material

CAS-Name: Silica, amorphous, fumed, crystalline free, CAS-No. 112945-52-5

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Administration / exposure

Route of administration

oral: feed

Duration of treatment / exposure

Exposure period: 6 months
Premating exposure period (males): 4.5 months
Premating exposure period (females): 4.5 months
Duration of test: 6 months

Frequency of treatment

daily

Details on study schedule

Number of generation studies: 1

Doses / concentrations

497 mg/kg bw (m); 509 mg/kg bw (f)

Basis

No. of animals per sex per dose

5 instead of 20 females, 1 male per 5 females

Control animals

yes

Examinations

Any other information on materials and methods incl. tables

Screening test: Parents (40 m / 40 f), treatment started at a mean weight of 90 - 110 g; mating procedure (14 d): 5 treated and 5 control females (mated to 1 male, resp.) after 4 1/2 months of exposure. The test-substance dose was adjusted to the body-weight gain. Hematology carried out in 5 animals of each group prior to exposure, each month and at the end of the study. Histopathology only in parent animals. Pups were examined for external appearance and development.
Note: As compared to current standards, number of pregnant animals was too low (5 instead of 20

females), mating ratio was 1:5 instead of 1:2; only 1 male used per treated and control group; one dose tested, not at the limit as recommended in the OECD guideline 415.

Results and discussions

Effect levels

Endpoint	NOAEL
Generation	P
Sex	
Effect level	497 mg/kg bw/day
Basis for effect level / Remarks	
Endpoint	NOAEL
Generation	F1
Sex	
Effect level	497 mg/kg bw/day
Basis for effect level / Remarks	

Observations: offspring

Remarks on results including tables and figures

Result: negative
Parents: No clinical symptoms; no mortality, no abnormalities in body-weight gain and feed consumption, no haematological findings.
In pups during lactation [total: 45 and 37 (control), resp.], no behavioral or developmental or structural abnormalities. --------

General information

Reference substance name silicon dioxide

EC inventory

EC number 231-545-4 **CAS number** 7631-86-9

EC name silicon dioxide

Molecular formula O2Si

No EC information available

Justification not applicable

Reference substance information

CAS information

CAS number 7631-86-9

IUPAC name

IUPAC name dioxosilane

Description

Silicas are found under CAS No.7631-86-9, i.e. this is the old and general CAS Number listed in the EINECS which includes all forms of silicas (e.g. also crystalline and natural forms, independent of its form and method of preparation including by-products). The following polymorph forms are not

considered here: Natural forms of amorphous silica like diatomaceous earth, especially flux-calcined diatomaceous earth, or silica fume - a byproduct of producing silicon metal or ferrosilicon alloys - may contain impurities or may contain crystalline impurities. As the polymorphs of silica differ in their hazards to human health, it is essential to distinguish carefully between crystalline silica and crystalline-free or amorphous silica forms. Only the synthetic amorphous silicas are subject of this dossier.
The new CAS Numbers distinguish between the different types of synthetic amorphous silicas:
Pyrogenic synthetic amorphous silica CAS: 112945-52-5
Precipitated synthetic amorphous silica CAS: 112926-00-8.

Synonyms

Name Silica

Name Highly dispersed silica

Name Hochdisperses Silizium Dioxid (Deutsches Arzneibuch)

Name Light anhydrous silicic acid (Japanese Pharmacopoeia)

Name Precipitated silica

Name Pyrogenic (fumed) amorphous silica

Name Silica; amorphous, fumed, crystalline-free

Name Siliziumdioxid

Related CAS information

CAS name	CAS number	Justification
Silica, amorphous, fumed, crystalline-free	112945-52-5	other: see description above

CAS name	CAS number	Justification
Silica, amorphous, fumed, crystalline-free	112945-52-5	other: see description above
Silica gel and precipitated silica, crystalline-free	112926-00-8	other: see description above

DSL Category: Inorganics

Molecular and structural information

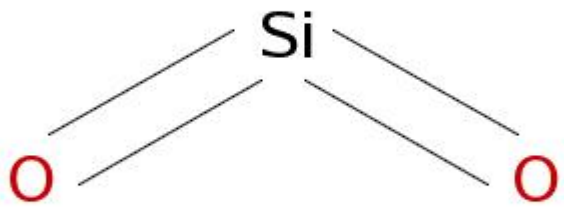
Molecular formula O2Si

Molecular weight range 60.0843

SMILES notation O=[Si]=O

InChI InChI=1/O2Si/c1-3-2

Structural formula



Remarks SiO2 aggregates and agglomerates

8. ANALYTICAL METHODS

9. RESIDUES IN FOOD AND FEEDINGSTUFFS

10. EFFECTIVENESS AGAINST TARGET ORGANISMS

11. GUIDANCE ON SAFE USE

12. LITERATURE SEARCH

13. ASSESSMENT REPORTS

14. INFORMATION REQUIREMENTS