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# DOSSIER ON SILICON DIOXIDE (NM 200) - PART 1 -

**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.*

*This document is only available in PDF format.*

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**OECD Environment, Health and Safety Publications**

**Series on the Safety of Manufactured Nanomaterials**

**No. 51**

# DOSSIER ON SILICON DIOXIDE (NM 200)
# - PART 1 -

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

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

***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)*

**ABOUT THE OECD**

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in eleven different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents;** and **Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/chemicalsafety/).

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

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

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# PREAMBLE

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

Before launching the Testing Programme, the WPMN first identified a broad list of possible nanomaterials, and the list was later adjusted to a final selection of eleven MNs for 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.

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[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 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 and to Nathalie Thieriet and 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

PREAMBLE....7
FOREWORD....9
ACKNOWLEDGMENTS....10
1. GENERAL INFORMATION....14
1.1 Identification....14
1.2 Composition....14
1.3 Identifiers....14
1.4 Analytical information....14
1.5 Joint submission....15
1.6 Sponsors....15
1.7 Suppliers....15
1.8 Recipients....15
1.9 Product and process oriented research and development....15
2. CLASSIFICATION AND LABELLING....15
2.1 GHS....15
2.2 DSD - DPD....15
3. MANUFACTURE, USE AND EXPOSURE....15
3.1 Technological process....15
3.2 Estimated quantities....15
3.3 Form in the supply chain....15
3.4 Identified uses and exposure scenarios....15
3.5 Uses advised against....15
3.6 Waste from production and use....15
3.7 Exposure estimates....15
3.8 Biocidal information....15
3.9 Application for authorisation of uses....15
4. PHYSICAL AND CHEMICAL PROPERTIES....16
4.0 Stability and homogeneity....16
4.0.1 Homogeneity....16
4.0.2 Stability, short-term (Assessment of Shipment Conditions)....20
4.1 Appearance....23
4.4 Density....25
4.5 Particle size, size distribution....26
4.6 Vapour pressure....38
4.7 N-octanol-water partition coefficient....38
4.8 Water solubility, hydrophilicity, dispersibility....38
4.9 Solubility in organic solvents / fat solubility....41
4.10 Surface tension....41
4.11 Flash point....41
4.12 Auto flammability....41

4.13 Flammability ..... 41
4.14 Explosiveness ..... 41
4.15 Oxidising properties ..... 42
4.16 Oxidation reduction potential ..... 42
4.17 Stability in organic solvents and identity of relevant degradation products ..... 42
4.18 Storage stability and reactivity towards container material ..... 42
4.19 Stability: thermal, sunlight, metals ..... 42
4.20 pH ..... 42
4.21 Dissociation constant ..... 42
4.22 Viscosity ..... 42
4.23 Additional physico-chemical information ..... 42
4.24 Agglomeration/aggregation ..... 47
4.25 Crystalline phase ..... 49
4.27 Aspect ratio, shape ..... 57
4.28 Specific surface area ..... 58
4.29 Zeta potential ..... 65
4.30 Surface chemistry ..... 69
4.31 Dustiness ..... 71
4.32 Porosity ..... 78
4.35 Radical formation potential ..... 80
5. ENVIRONMENTAL FATE AND PATHWAYS ..... 81
5.1 Stability ..... 81
5.1.1 Phototransformation in air ..... 81
6. ECOTOXICOLOGICAL INFORMATION ..... 81
6.1 Aquatic toxicity ..... 81
6.1.1 Short-term toxicity to fish ..... 81
6.1.2 Long-term toxicity to fish ..... 85
6.1.3 Short-term toxicity to aquatic invertebrates ..... 85
7. TOXICOLOGICAL INFORMATION ..... 90
7.1 Toxicokinetics, metabolism and distribution ..... 90
7.1.1 Basic toxicokinetics ..... 90
7.2 Acute Toxicity ..... 99
7.2.1 Acute toxicity: oral ..... 99
7.2.2 Acute toxicity: inhalation ..... 105
7.3 Irritation / corrosion ..... 110
7.3.1 Skin irritation / corrosion ..... 110
7.3.2 Eye irritation ..... 117
7.4 Sensitisation ..... 120
7.5 Repeated dose toxicity ..... 120
7.5.1 Repeated dose toxicity: oral ..... 120
7.5.2 Repeated dose toxicity: inhalation ..... 132
7.5.3 Repeated dose toxicity: dermal ..... 147
7.5.4 Repeated dose toxicity: other routes ..... 147
7.6 Genetic toxicity ..... 147

7.6.1 Genetic toxicity in vitro....147
7.6.2 Genetic toxicity in vivo....183
7.7 Carcinogenicity....198
7.8 Toxicity to reproduction....198
7.8.1 Toxicity to reproduction....198
8. ANALYTICAL METHODS....208
9. RESIDUES IN FOOD AND FEEDINGSTUFFS....208
10. EFFECTIVENESS AGAINST TARGET ORGANISMS....208
11. GUIDANCE ON SAFE USE....208
12. LITERATURE SEARCH....208
13. ASSESSMENT REPORTS....208
14. INFORMATION REQUIREMENTS....208

# Substance: NM-200 silicon dioxide

# 1. General Information

## 1.1 Identification

### Substance identification

**Chemical name** NM-200 silicon dioxide

## 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-200 precipitated SAS |
| **Method used** | X-ray diffraction |
| | NM-200 XRD.pdf / 84.99 KB |
| **Remarks** | See also chapter 4.25 crystalline phase |
| **Analysis type** | Infrared spectroscopy (IR ATR) |
| **Tested substance** | NM-200 precipitated SAS |
| **Method used** | Infrared spectroscopy (IR-Spectroscopy) |
| | NM-200 IR.pdf / 78.53 KB |
| **Remarks** | Typical peaks for silanol groups of the particle surface see spectra. |
| **Analysis type** | NMR |
| **Tested substance** | NM-200 precipitated SAS |
| **Method used** | NMR Spectroscopy |

| | |
|---|---|
| | NM-200 NMR.pdf / 105.11 KB |
| **Remarks** | Interpretation of the spectrum: see spectra |

**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

## 4.0 Stability and homogeneity

### 4.0.1 Homogeneity

### *Endpoint study record: Homogeneity inter and intra vial by DLS by CEA*

#### Administrative Data

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

**Study result type** experimental result

#### Data source

#### Data access

other: owner: NANOGENOTOX

#### Materials and methods

#### Methods

DLS

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

General description of scientific back ground. Dynamic Light Scattering (DLS), also called Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS), is a technique of characterization of colloidal systems based on the scattering of visible light resulting from the difference in refractive index between the dispersed colloids and the dispersion medium. The method may be applied for sizing particles suspended in a liquid in the range from about 0.6 nm to about 6 μm depending on the optical properties of the material and medium.The principle in DLS is measurement of fluctuations in laser light scattered by vibrating particles suspended in a liquid as function of time. The vibration is due to Brownian motion caused by collision with solvent molecules of the liquid. The Brownian motion varies as a function of particle size and causes variation in the intensity of scattered light as function of time. A correlator compares the signal measured at a time $t_0$ with different very short time delays dt (autocorrelation). As the particles move, the correlation between $t_0$ and subsequent dt signals decreases with time, from a perfect correlation (1) at $t_0$, to a complete decorrelation (0) at infinite time (order of milliseconds). In the case of big particles, the signal changes slowly and the correlation persists for a long time, whereas small particles have high Brownian movement causing rapid decorrelation. A DLS instrument measures the velocity of Brownian motion, defined by the translational diffusion coefficient D of the particles. The particle size, or more precisely its hydrodynamic diameter dh, is then estimated using the Stokes-Einstein equation assuming spherical shape.It should be noted that even if a particle is really spherical, the spherical DLS size is fundamentally different from the physical spherical size. The hydrodynamic size includes the double-layer of highly polarized water molecules around the physical particle. When the particle morphology is highly non-spherical, the hydrodynamic size should be understood as the equivalent hydrodynamic spherical size. Establishment of mean hydrodynamic size and size distributions (intensity, number, volume) is reached by DTS software algorithms, by fitting the correlation function in the data treatment.The detailed description of the protocol used for DLS measurments can be found in the attached file: SOP for DLS.doc .

## Details on methods and data evaluation

Measurements are performed at ambient temperature according to the procedure appropriate for each type of apparatus. Sample properties such as material and dispersant refractive indices and viscosity are entered in the software for analysis. Number and duration of run and optical configuration are automatically optimized by the software for Malvern apparatus. For Cordouan apparatus, 15 runs of 60s are performed. On the measurments: DLS measurements can be performed in disposable polystyrene cuvettes (optical path 1 cm, volume 1 mL) or alternatively glass cuvettes (at NRCWE) or in semi micro polystyrene disposable cuvettes (optical path 1 cm, volume 500 µL) or in clear disposable zeta cells DTS1061 just before zeta potential measurements (at CEA). The measurements are repeated 3 (CEA) or 6 (NRCWE) times with automatic determination of duration and number of runs, and averaged. The repeated analyses are conducted to enable omission of measurements with poor correlation data or abnormal solutions to the correlation function (must be carefully considered). The following standard procedure is recommended as the general approach for DLS measurement of NM dispersions:

• Turn on the computer and DLS instrument

• Allow the instrument to warm up according to the manufacturer's recommendation (30 min)

• Optional: Complete viscosity measurement using the SV-10 Vibro Viscometer mounted with the 10 ml flow-reactor placed in a thermostated water jacket. The measured dynamic viscosity is used as input data for the specific dispersion measured in the DTS software.

• Upload the DTS software and the "Measurement" window for entering material specific data on dispersion medium and test material as well as specific analytical settings :o Refractive index and absorption values for dispersant and NMo Temperature conditions (25°C) and equilibration time for measuremento The General purpose model is selected for initial evaluation of data and is the most generic model for calculation of size.

• Select a sample cuvette and ensure that it does not contain dust, nor defects or scratches in the measurement area of the cuvette. Some producers have been found to deliver cuvettes with scratches or folding structure in the measurement area at one side of the cuvette. Dust may be cleaned out by rinsing the cuvette in dispersion medium.

• Fill in a suitable volume of the dispersion into a suitable measurement cuvette using a pipette

• Place the sample cuvette in the sample holder in the DLS instrument• Run analysis (click "play" on the measurement window)

• The size analysis may be immediately accepted if the DTS Expert advice denotes the result quality as "Good". If the result is not of good quality, the sample should be further analyzed for presence of dust, cuvette errors, large particles, sedimentation, wall-deposition etc.

• If the sample contains particles with large spread in size distribution, one may consider filtering the sample through different syringe filters to investigate presence of small nm-size particles. Small nm-size particles may not be fully resolved when larger particles are present due to the large drop (106 per factor of ten in size ratio) in scattered light intensity with size.

• If parameters such as refractive indexes, absorption coefficient or viscosity were wrong or unknown at the measurement time, the correction can be made afterwards using the command Edit (right click on the measurement) in the DTS software.The viscosity considered for measurement is generally the one of pure water, 0.8872 cP, but the data can be corrected afterwards for the values measured.At CEA, the viscosity of water is considered for all samples prepared without addition of BSA or in the pH-adjusted protocol.

### *Used Protocols*

NANOGENOTOX SOP FOR DISPERSION

### Used Protocols: attached files

| | |
|---|---|
| **Attached document** | SOP for DLS.doc / 1.21 MB (application/msword): SIAR |
| **Remarks** | SOP for DLS measurments and data treatment |

## Data gathering

### Instruments

♣Chemicals and equipment
♣Test material or chemical
♣Dispersion medium
♣Ultrasonic probe equipped with a standard 13 mm disruptor horn
♣Dynamic Light Scattering apparatus
♣Viscosimeter (Malvern Inc. Optional for measurement of true viscosities)
♣Pipette and pipette tips
♣Syringes and syringe filters or filter paper
♣Zetasizer NanoZS from Malvern Instruments

DLS measurements rely on non-invasive back scatter (NIBS®) technology developed by Malvern Instruments, in which the signal is detected at 173°. The signal is treated by a digital correlator, and transmitted to the computer. DTS software enables the fitting of correlation data either by a monomodal mode, called the cumulant analysis (as defined by ISO 13321 Part 8) to obtain a mean size (Z-average diameter) and a polydispersity index (PDI), or by a multiple exponential known as the CONTIN method to obtain a distribution of particle sizes. Sample preparation: Dispersions for analysis are prepared by mixing particulate material into a dispersion medium. A sub-sample of a suitable concentration is added to suitable measurement cuvettes. Dispersions are typically produced by sonication in a dispersion medium; SOPs were developed for dispersing the NMs, see e.g. www.nanogenotox.eu. The dispersion medium must be filtrated before use to avoid any dust contamination. This can be done by using syringe filters or filter paper with high efficiency. Usually filters with a 0.2 to 0.45 μm pore-size are sufficient for filtration of dispersion media.The concentration required for analysis depends e.g. on the relative refractive index between particles and dispersion medium, the particle size and polydispersity and the sample absorption. Malvern apparatus is designed to measure samples over a large range of concentration and size of particles. Specifications of sample properties (concentration range, size of nanoparticles, medium) are found in the documentation from Malvern Instrument on their website. The dispersion must be stable during the measurement.

### Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### State of test material

other: fluffy powder in the dispersion

## Overall remarks, attachments

### Overall remarks

Strategy Description:
• Partners received the samples in vials and the homogeneity both within vials and between vials were assessed by DLS measurements on aqueous suspensions in the best dispersed state.
• The Nanogenotox sample preparation protocol was applied (see the attached document with SOP for dispersion).
• The homogeneity within a vial was assessed by DLS measurements performed on a series of samples. These were prepared by the same operator, under the same conditions and from the same vial, and thus illustrate both the homogeneity within one vial and the reproducibility of the sample preparation by a given operator.
• The homogeneity between vials was evaluated by measurements on a series of samples from different vials of a given NM, prepared by the different laboratories. This would quantify both the variability between vials of the given NM, and between sample preparations from the different laboratories.
• The following parameters were compared:o measurement results on independent samples from the same vial, o measurement results on samples from different vials.
• When several samples from one vial were tested, mean values with standard deviations are reported.
• The data reported are:
- Z-average particle diameter (Nobbmann et al., 2007) (Z-average mean value, which corresponds to the particle size diffusing with the highest intensity)
- polydispersity index (PdI)(monomodal distribution), calculated using the cumulant method for Malvern apparatus.
• The position of the main peak of the intensity size distribution was modelled with a multimodal analysis. For Malvern apparatus, the CONTIN method was used and the width of the main peak is also reported.
Results:
• Ultra-pure water dispersion (intra vial study)
Z-average (nm): 207.1 ± 12.3, PdI: 0.390 ± 0.041

### Attached full study report

Draft D4.5 ZETA DLS SAXS analysis.pdf / 2.03 MB (application/pdf): ENV/JM/MONO(2015)14/ANN5

## Applicant's summary and conclusion

### Conclusions

The variablity intra-vial observed is about 6-10%

## 4.0.2 Stability, short-term (Assessment of Shipment Conditions)

### *Endpoint study record: Stability, short-term_NM-200 suspension for DLS*

#### Administrative Data

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

**Study result type** experimental result

#### Materials and methods

#### Methods

DLS

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

General description of scientific back ground Dynamic Light Scattering (DLS), also called Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS), is a technique of characterization of colloidal systems based on the scattering of visible light resulting from the difference in refractive index between the dispersed colloids and the dispersion medium. The method may be applied for sizing particles suspended in a liquid in the range from about 0.6 nm to about 6 μm depending on the optical properties of the material and medium.The principle in DLS is measurement of fluctuations in laser light scattered by vibrating particles suspended in a liquid as function of time. The vibration is due to Brownian motion caused by collision with solvent molecules of the liquid. The Brownian motion varies as a function of particle size and causes variation in the intensity of scattered light as function of time. A correlator compares the signal measured at a time t0 with different very short time delays dt (autocorrelation). As the particles move, the correlation between t0 and subsequent dt signals decreases with time, from a perfect correlation (1) at t0, to a complete decorrelation (0) at infinite time (order of milliseconds). In the case of big particles, the signal changes slowly and the correlation persists for a long time, whereas small particles have high Brownian movement causing rapid decorrelation.A DLS instrument measures the velocity of Brownian motion, defined by the translational diffusion coefficient D of the particles. The particle size, or more precisely its hydrodynamic diameter dh, is then estimated using the Stokes-Einstein equation assuming spherical shape.It should be noted that even if a particle is really spherical, the spherical DLS size is fundamentally different from the physical spherical size. The hydrodynamic size includes the double-layer of highly polarized water molecules around the physical particle. When the particle morphology is highly non-spherical, the hydrodynamic size should be understood as the equivalent hydrodynamic spherical size. Establishment of mean hydrodynamic size and size distributions (intensity, number, volume) is reached by DTS software algorithms, by fitting the correlation function in the data treatment.

#### Details on methods and data evaluation

Measurements are performed at ambient temperature according to the procedure appropriate for each type of apparatus. Sample properties such as material and dispersant refractive indices and viscosity are entered in the software for analysis. Number and duration of run and optical configuration are automatically optimized by the software for Malvern apparatus. For Cordouan apparatus, 15 runs of 60s are performed.On the measurments:DLS measurements can be performed in disposable polystyrene cuvettes (optical path 1 cm, volume 1 mL) or alternatively glass cuvettes (at NRCWE) or in semi micro polystyrene disposable cuvettes (optical path 1 cm, volume 500 µL) or in clear disposable zeta cells

DTS1061 just before zeta potential measurements (at CEA). The measurements are repeated 3 (CEA) or 6 (NRCWE) times with automatic determination of duration and number of runs, and averaged. The repeated analyses are conducted to enable omission of measurements with poor correlation data or abnormal solutions to the correlation function (must be carefully considered). The following standard procedure is recommended as the general approach for DLS measurement of NM dispersions:• Turn on the computer and DLS instrument• Allow the instrument to warm up according to the manufacturer's recommendation (30 min)
• Optional: Complete viscosity measurement using the SV-10 Vibro Viscometer mounted with the 10 ml flow-reactor placed in a thermostated water jacket. The measured dynamic viscosity is used as input data for the specific dispersion measured in the DTS software.
• Upload the DTS software and the "Measurement" window for entering material specific data on dispersion medium and test material as well as specific analytical settings :
• Refractive index and absorption values for dispersant and NMo Temperature conditions (25°C) and equilibration time for measuremento The General purpose model is selected for initial evaluation of data and is the most generic model for calculation of size.
• Select a sample cuvette and ensure that it does not contain dust, nor defects or scratches in the measurement area of the cuvette. Some producers have been found to deliver cuvettes with scratches or folding structure in the measurement area at one side of the cuvette. Dust may be cleaned out by rinsing the cuvette in dispersion medium.
• Fill in a suitable volume of the dispersion into a suitable measurement cuvette using a pipette • Place the sample cuvette in the sample holder in the DLS instrument
• Run analysis (click "play" on the measurement window)
• The size analysis may be immediately accepted if the DTS Expert advice denotes the result quality as "Good". If the result is not of good quality, the sample should be further analyzed for presence of dust, cuvette errors, large particles, sedimentation, wall-deposition etc.
• If the sample contains particles with large spread in size distribution, one may consider filtering the sample through different syringe filters to investigate presence of small nm-size particles. Small nm-size particles may not be fully resolved when larger particles are present due to the large drop (106 per factor of ten in size ratio) in scattered light intensity with size.
• If parameters such as refractive indexes, absorption coefficient or viscosity were wrong or unknown at the measurement time, the correction can be made afterwards using the command Edit (right click on the measurement) in the DTS software.The viscosity considered for measurement is generally the one of pure water, 0.8872 cP, but the data can be corrected afterwards for the values measured. At CEA, the viscosity of water is considered for all samples prepared without addition of BSA or in the pH-adjusted protocol.

## Data gathering

### Instruments

Chemicals and equipment
♣ Test material or chemical
♣ Dispersion medium
♣ Ultrasonic probe equipped with a standard 13 mm disruptor horn
♣ Dynamic Light Scattering apparatus
♣ Viscosimeter ( Malvern Inc. Optional for measurement of true viscosities
♣ Pipette and pipette tips
♣ Syringes and syringe filters or filter paperZetasizer NanoZS from Malvern InstrumentsDLS measurements rely on non-invasive back scatter (NIBS®) technology developed by Malvern Instruments, in which the signal is detected at 173°. The signal is treated by a digital correlator, and transmitted to the computer. DTS software enables the fitting of correlation data either by a monomodal mode, called the cumulant analysis (as defined by ISO 13321 Part 8) to obtain a mean size (Z-average diameter) and a

polydispersity index (PDI), or by a multiple exponential known as the CONTIN method to obtain a distribution of particle sizes.Sample preparation:Dispersions for analysis are prepared by mixing particulate material into a dispersion medium. A sub-sample of a suitable concentration is added to suitable measurement cuvettes. Dispersions are typically produced by sonication in a dispersion medium; SOPs were developed for dispersing the NMs, see e.g. www.nanogenotox.eu. The dispersion medium must be filtrated before use to avoid any dust contamination. This can be done by using syringe filters or filter paper with high efficiency. Usually filters with a 0.2 to 0.45 μm pore-size are sufficient for filtration of dispersion media.The concentration required for analysis depends e.g. on the relative refractive index between particles and dispersion medium, the particle size and polydispersity and the sample absorption. Malvern apparatus is designed to measure samples over a large range of concentration and size of particles. Specifications of sample properties (concentration range, size of nanoparticles, medium) is found in the documentation from Malvern Instrument on their website. The dispersion must be stable during the measurement.

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### State of test material

other: fluffy powder in dispersion

## Results and discussions

### Strategy description

• Partners received the samples in vials and the homogeneity both within vials and between vials were assessed by DLS measurements on aqueous suspensions in the best dispersed state.
• The Nanogenotox sample preparation protocol was applied (see the attached document with SOP for dispersion).
• The homogeneity within a vial was assessed by DLS measurements performed on a series of samples. These were prepared by the same operator, under the same conditions and from the same vial, and thus illustrate both the homogeneity within one vial and the reproducibility of the sample preparation by a given operator.
• The homogeneity between vials was evaluated by measurements on a series of samples from different vials of a given NM, prepared by the different laboratories. This would quantify both the variability between vials of the given NM, and between sample preparations from the different laboratories.
• The following parameters were compared:o measurement results on independent samples from the same vial, o measurement results on samples from different vials.
• When several samples from one vial were tested, mean values with standard deviations are reported. • The data reported are:
• Z-average particle diameter (Nobbmann et al., 2007) (Z-average mean value, which corresponds to the

particle size diffusing with the highest intensity)o polydispersity index (PdI)(monomodal distribution), calculated using the cumulant methodfor Malvern apparatus.
• The position of the main peak of the intensity size distribution was modelled with a multimodal analysis. For Malvern apparatus, the CONTIN method was used and the width of the main peak is also reported.

**Overall remarks, attachments**
**Overall remarks**

Ultra-pure water dispersion (intra vial study)
Z-average (nm): 207.1 ± 12.3, PdI: 0.390 ± 0.041

## 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 of AT | **Year** | 2009 |
| **Title** | Particle Size of precipitated synthetic amorphous silica | | |
| **Bibliographic source** | unpublished report | | |
| **Testing laboratory** | | **Report no.** | |
| **Owner company** | Rhodia Operations | | |
| **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-200

**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-200

### Results and discussion

**Physical state at 20ºC and 1013 hPa**
solid

**Form**
powder

**Colour**
white

**Odour**
odourless

**Substance type**
inorganic

## *Endpoint study record: Appearance*

### Administrative Data

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

### Results and discussion

**Physical state at 20ºC and 1013 hPa**
solid

**Form**
powder

**Colour**
white

**Odour**
odourless

**Substance type**
inorganic

**Overall remarks, attachments**

**Overall remarks**
fluffy powder

## 4.4 Density

### *Endpoint study record: Density/Tapped 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**
Tapped 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. 182 g/L

**Temp.**

### Overall remarks, attachments

### Overall remarks

Tapped density of NM-200 is 182g/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** | | | |
| **Testing laboratory** | Technische Universität Dresden, Faculty of Mechanical Engineering, Institute of Process Engineering and Environmental Technology | **Report no.** | |
| **Owner company** | Rhodia Operations | | |
| **Company study no.** | | **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-200 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-200. 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 spectrometerThe 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 height 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 480 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

### Confidential details on test material

NM-200

### Any other information on materials and methods incl. tables

| | X16,3 [µm] | X50,3 [µm] | X84,3 [µm] | X99,3 [µm] | copt. [%] |
|---|---|---|---|---|---|
| 1. Run | 293.48 | 469.25 | 694.02 | 994.86 | 2.60 |
| 2. Run | 303.68 | 485.71 | 712.52 | 994.99 | 1.80 |
| 3. Run | 297.30 | 486.96 | 728.69 | 1145.38 | 3.00 |
| mean | 298.15 | 480.64 | 711.74 | 1045.08 | 2.47 |

Median diameters, optical concentrations and standard deviations of NM-200 measured with the HELOS-GRADIS-System

## Results and discussions

### Mean diameter

≥480 µm

### Particle size

**Percentile** D50

**Mean** ≥ 480 µm

**St. dev.**

### Particle size distribution at different passages

**No.** #1

**Size** ≥ 469 µm

**Distribution**

**No.** #2

**Size** ≥ 486 µm

**Distribution**

**No.** #3

**Size** ≥ 487 µm

**Distribution**

### Overall remarks, attachments

#### Overall remarks

The median particle diameter weighted by volume amounts to 480 µm.

#### Attached background material

**Attached document** NM-200 PSD.pdf / 74.98 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 480 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** | |
| **Title** | Determination of particle sizes in dispersion of water | | |
| **Bibliographic source** | Department of AT | | |
| **Testing laboratory** | | **Report no.** | |
| **Owner company** | Rhodia Operations | | |
| **Company study no.** | Department of AT | **Report date** | |

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-200

## 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: 0.3wt% Liquid phase: WaterDispersion: Ultrasonic (Vibracell), 240 sec, energy input:600W

### 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.

### Data gathering

### Instruments

Equipment: Malvern Mastersizer

## 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

≥ 15.22 µm

### Particle size

**Percentile** D50

**Mean** ≥ 15.22 µm

**St. dev.**

### Overall remarks, attachments

### Attached background material

**Attached document** NM-200 PSD 2.pdf / 59.63 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5

**Remarks**

### Applicant's summary and conclusion

### Conclusions

Mean particle size diameter of aggregates in dispersion of water are > 15 µm.

### 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** | |
| **Title** | Primary particles from TEM | | |
| **Bibliographic source** | Department of AT | | |
| **Testing laboratory** | | **Report no.** | |
| **Owner company** | Rhodia Operations | | |
| **Company study no.** | Department of AT | **Report date** | |

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-200

### Materials and methods

### Test guideline/method

**Qualifier** no guideline followed

**Guideline**

**Deviations**

### Methods

TEM

### 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: 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:Spatula of material, add 5 ml Aqua, disperse 5-8 minutes ultrasonic at 50W.

## Results and discussions

### Mean diameter

> 5 — < 20 nm

## 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-200 TEM.pdf / 72.57 KB (application/pdf) : ENV/JM/MONO(2015)14/ANN5

#### Remarks

## Applicant's summary and conclusion

### Conclusions

Agglomerated Silica; the elementary particle size varies from 5 to 20 nm with an average size mainly

around 10 -15 nm.

### Executive summary

## *Endpoint study record: Particle size, size distribution by TEM_IMC_BAS*

### Administrative Data

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

## *Endpoint study record: Particle size, size distribution by TEM_Quantitative_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** | 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** | | **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.o 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-200 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. 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 expirience suggests that this approach is easier than the alternative based on glow discharging EM-grids with air to introduce negative charges and subsequent $Mg^{2+}$ treatment, introducing positive charges). For TEM measurments the suspended NM-200 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) |
| **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

Not stated. The method was developed to have a standardised TEM method.

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### 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

# *Endpoint study record: Size distribution and anitensity avaraged mean size of aggregates by DLS by CEA*

## Administrative Data

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

## Data source

### Cross-reference to same study

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

**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-200

**Test material identity**
**Identifier** CAS number
**Identity** 7631-86-9

**State of test material**
other: fluffy powder in dispersion

## 4.6 Vapour pressure

## 4.7 N-octanol-water partition coefficient

### *Endpoint summary: N-octanol-water partition coefficient*

**Administrative Data**

**Short description of key information**
Not applicable. NM-200 is not soluble in n-octanol. Note: This specific endpoint characteristics is inherent to the substance and is not linked to a specific lot.

## 4.8 Water solubility, hydrophilicity, dispersibility

### *Endpoint study record: Water solubility, hydrophilicity, dispersibility.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)

## Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | other: Study summary | | |
| **Author** | Head of laboratory | **Year** | |
| **Title** | Spectrophotometric determination of silicate according to Motomizu | | |
| **Bibliographic source** | | | |
| **Testing laboratory** | | **Report no.** | |
| **Owner company** | Rhodia Operations | | |
| **Company study no.** | | **Report date** | |

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

OECD Sponsorship programme for the testing of Manufactured Nanomaterials NM-200

## Materials and methods

### Test guideline/method

**Qualifier** according to

**Guideline** other guideline: Motomizu, Spectrophotometric Determination of Silicate in water with Molybdate and Malachite Green

**Deviations**

### Type of method

flask method

### Principles of method if other than guideline

Spectrophotometric determination of silicate according to Motomizu. Orthosilicic acid reacts with molybdate anions under strong acid conditions to form dodecamolybdatosilicateanions $[SiMo_{12}O_{40}]^{4-}$ with malachite a green complex are formed. In the presence of a protective colloid (5 wt%, aqueous PVA solution) photometric determination at a wavelength of 595 nm is performed

### Details on methods and data evaluation

Motomizu S., Oshima M., Ojima Y., Spectrphotometric Determination of Silicate in Water with Molybdate and Malachite Green, Anal. Sci. 5, 1989. S 85-88

***Used Protocols***

Method: Spectrophotometric determination of silicate according to Motomizu.
Equipment: Spekol 1100, Analytik JenaBuffer system: 50.00 ml Tris(hydroxymethyl)aminomethan (0.1 mol/l) 35.45 ml $HCl^-$ Solution (0.1 mol/l) 14.55 ml pure water Electrolyte: 0.1 mol/l NaCl pH 7.1 - 7.4 Temperature: 37 °C Flask: PMP (PolyMethylPenten); avoid contact with glass; check silicate content of all used chemicals; double de-ionized water (Seralpur Pro CN 90, 0.055 µS/cm) is used Separation $SiO_2$ from the solved orthosilicic acid: 0.2 µm filter (Schleicher & Schuell)

## 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-200.

### Confidential details on test material

The test substance is equivalent to NM-200.

## Results and discussions

***Details on results***

The saturation concentration for NM-200 has been determined to: 2.4 ± 0.03 mmol/l. Time to plateau: 4.5 h

## Overall remarks, attachments

### Overall remarks

For almost all SASs, the saturation concentration was reached in a few hours. The saturation concentrations [M]s, tot for all analyzed SAS ranged from 1.9 to 2.5 mmol/l.

### Attached background material

**Attached document** NM-200 solubility.pdf / 23.46 KB (application/pdf): ENV/JM/MONO(2015)14/ANN7

**Remarks**

## Applicant's summary and conclusion

### Conclusions

The saturation concentrations [M],tot for test substance equivalent to NM-200 has been determined to 2.4 mmol/l.

## 4.9 Solubility in organic solvents / fat solubility

## 4.10 Surface tension

## 4.11 Flash point

## 4.12 Auto flammability

## 4.13 Flammability

### *Endpoint summary: Flammability*

**Administrative Data**

**Short description of key information**

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

## 4.14 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. NM-200 is not dust-explosive.Note: This specific endpoint characteristics is inherent to the substance and is not linked to a specific lot.

## 4.15 Oxidising properties

## 4.16 Oxidation reduction potential

### *Endpoint summary: Oxidation reduction potential*

**Administrative Data**

**Short description of key information**

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

## 4.17 Stability in organic solvents and identity of relevant degradation products

## 4.18 Storage stability and reactivity towards container material

## 4.19 Stability: thermal, sunlight, metals

## 4.20 pH

## 4.21 Dissociation constant

## 4.22 Viscosity

## 4.23 Additional physico-chemical information

### *Endpoint study record: 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**

**Data access**
other: owner: NANOGENOTOX

**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 or cooled with the same rate. The temperature is measured for both sample and reference, and the difference is calculated. Most transformations such as phase transitions, melting, crystallization, decomposition etc. are either endothermic or exothermic; that is they either require or release energy. 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-200

### 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

# *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

## 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 toolin electron microscopes. The analysis is based on the fact that when hitting a material with charged particles,

such as anelectron 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 againduring 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.

**Results and discussions**
**Results**

Na (ppm/wt %): 8800
Al (ppm/wt %): 4600
Si (wt %): 44.77
S (ppm/wt %): 8700
Ca(ppm/wt %): 0
O (wt %): 53.02

**Remarks on results including tables and figures**

no data on the instruments used.

**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.

## *Endpoint study record: composition by ICP_OES by*

**Administrative Data**

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

**Study result type** experimental result

**Materials and methods**

**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 a fast

estimate of the concentration of non-calibrated compounds in samples. The samples were screened for 68 elements (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-200 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 over night 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-200

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### Results and discussions

### Results

Impurities ranges found for NM-200Na >1mg/gMg> 10ug/gAl>100ug/gS >1mg/gK >50ug/gCa>100ug/gFe>50ug/gZr>10Ug/g

**Applicant's summary and conclusion**

### Conclusions

Major impurities which were found in NM-200 sample are : Na, S, Al, Ca, Fe, K, Zr and Mg.This finding confirms the XRD results.

## *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

### Data source

### 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 ofparticles 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 of combustible compounds, which could in fact all be structurallydifferent 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 aluminaa nd 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-200

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### Any other information on materials and methods incl. tables

The SOP used for TGA anlysis: Thermogravimetric Analysis (TGA): Renie Birkedal (NRCWE) based on NIST Recommended Practice Guide, Special Publication 960-19. General description: TGA is short for thermograviometric 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 allorganic material. In order to make sure e.g. all organic material is decomposed, it is recommended torun to 1000 °C. Materials and Chemicals: Powder (may be conditioned in a specific atmosphere and humidity conditions) Laboratory weigh (scale) Apparatus for thermogravimetric analysis ProcedureSample preparation:

♣ Weigh container.

♣ Fill container with material. Do not stamp it, as this may affect the evaporation/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. CNT samples are somewhat different. They are in many cases bundles, and these bundles may be different. 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 theweight 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, recommendes 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/minis 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, butthis 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 toobtain several measurements to calculate the mean and standard deviation of the weight loss andthe 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-200 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.Weight losses below 100 °C for NM-200, suggests that this samplecontain adsorbed water. The same samples also have a gradual mass-loss up to ca. 200 °C. This suggests presence of an organic compound, which may be functional coatings.Coating: Yes (or $H_2O$) Weight of coating (wt%): 3

## 4.24 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-200

## 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.

### Data gathering

#### Instruments

Equipment: Malvern Mastersizer

## 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: 0.3 wt% Liquid phase: WaterDispersion: Ultrasonic (Vibracell),240 sec., energy input: 600W

### Results and discussions

### Agglomerate/aggregate diameter
**Mean diameter**

≥ 15.2 µm

### Agglomerate/aggregate size

**Percentile** D50

**Mean** ≥ 15.22 µm

**St. dev.**

### Overall remarks, attachments

### Overall remarks

Aggregates represent the smallest, stable, non-dispersible particle units of three-dimensional structure for NM-200.

### Attached background material

**Attached document** NM-200 PSD 2.pdf / 59.63 KB (application/pdf) : SIAR

**Remarks**

### Applicant's summary and conclusion

### Conclusions

Mean particle size diameter of aggregates in dispersion of water are > 15 µm.

## *Endpoint study record: Agglomeration/aggregation by ...*

### Administrative Data

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

# 4.25 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** 26.06.2008

**Reliability** 1 (reliable without restriction)

## Data source

### Reference

**Reference type** study report

**Author** **Year** 2008

**Title** XRD Examination

**Bibliographic source**

**Testing laboratory** **Report no.**

**Owner company** Rhodia Operations

**Company study no.** **Report date**

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

OECD Sponsorship program for the testing of Manufactured Nanomaterials NM-200.

## 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.

## 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.

### Attached background material

**Attached document** NM-200 XRD.pdf / 84.99 KB (application/pdf) : SIAR

**Remarks**

### Applicant's summary and conclusion

### Conclusions

NM-200 is fully amorphous; no crystalline structure can be determined.

## *Endpoint study record: Crystalline phase by XRD by*

**Administrative Data**

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

**Study result type** experimental result

### Data source

### Data access

other: owner: NANOGENOTOX

### 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 2theta, 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 theScherrer 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 canbe 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 is added 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 equation.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 Advanceddiffractometer 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 fixed divergence slit 0.2°. The analyses were made in the stepping mode stepping 0.02 degree 2 theta per second 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-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 2 theta persecond 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 bedocumented 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 radianangle.

### Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### 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 sampleholder. 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 withvacuum 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 sampleholder. Then the powder sample was topped on the sample holder and vacuum grease. The mostimportant disadvantage of this procedure is a small shift of the zero point, as the sample is notentirely in the correct position.

## Results and discussions

### Common name

The NM-200 is amorphous.

### Remarks on results including tables and figures

impurities detected in NM-200:

| Content | number of time measured | vial 0072 | vial 0441 |
|---|---|---|---|
| Amorphous | 0 | | |
| Amorphous + $Na_2SO_4$ | 3 | 2 | 1 |
| Amorphous + $Na_2SO_4$ | 4 | 3 | 1 |
| Amorphous + $Na_2SO_4$ +Boehmite ($Al_2O_4$) | 4 | 3 | 1 |

| | | | |
|---|---|---|---|
| total number | 7 | 5 | 2 |

### 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 of other 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 underinvestigation.

### 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-200 is amorphous however several crystalline impurities were detected impurities detected in NM-200:

| Content | number of time measured | vial 0072 | vial 0441 |
|---|---|---|---|
| Amorphous | 0 | | |
| Amorphous + $Na_2SO_4$ | 3 | 2 | 1 |
| Amorphous + $Na_2SO_4$ | 4 | 3 | 1 |
| Amorphous + $Na_2SO_4$ +Boehmite ($Al_2O_4$) | 4 | 3 | 1 |
| total number | 7 | 5 | 2 |

## *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

### Data access

other: owner: NANOGENOTOX

### 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 there by 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 Advanceddiffractometer 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-ray beam 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 2 theta pers econd 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.

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### 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-phase unless it has crystallized or it contains other crystalline (undesired) impurities.The Synthetic Amorphous Silica (SAS) samples were very difficult to mount in a standard sampleholder. 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 withvacuum grease in a single crystal Si low background sample holder.Measurements of empty sample holder with vacuum grease only showed an amorphous signal in the XRD 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 notentirely in the correct position.

## Results and discussions

### Common name

The NM-200 is amorphous.

### Remarks on results including tables and figures

impurities detected in NM-200:

| Content | number of time measured | vial 0072 | vial 0441 |
|---|---|---|---|
| Amorphous | 0 | | |

| | | | |
|---|---|---|---|
| Amorphous + $Na_2SO_4$ | 3 | 2 | 1 |
| Amorphous + $Na_2SO_4$ | 4 | 3 | 1 |
| Amorphous + $Na_2SO_4$ +Boehmite ($Al_2O_4$) | 4 | 3 | 1 |
| total number | 7 | 5 | 2 |

### 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 underinvestigation.

### 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

impurities detected in NM-200:

| Content | number of time measured | vial 0072 | vial 0441 |
|---|---|---|---|
| Amorphous | 0 | | |
| Amorphous + $Na_2SO_4$ | 3 | 2 | 1 |
| Amorphous + $Na_2SO_4$ | 4 | 3 | 1 |
| Amorphous + $Na_2SO_4$ +Boehmite ($Al_2O_4$) | 4 | 3 | 1 |
| total number | 7 | 5 | 2 |

## 4.27 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** | Precipitated 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

### Overall remarks, attachments

### Attached background material

**Attached document** NM-200 Shape.pdf / 40.85 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5

**Remarks**

## 4.28 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: Areameter by Coulter

### 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** 220 m²/g

**Standard deviation**

**Applicant's summary and conclusion**

### Conclusions

Surface BET DIN ISO 9277 adsorption of nitrogen at 77.4 K for NM-200 has been determined to: 220 $m^2/g$

## *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**

**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, 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 socalled 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 th eequilibrium 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).Smaple preparation no special 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-200

## Test materials

## Test material equivalent to submission substance identity

yes

## Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

## 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 was compared with manufacturers data.
BET (manufacturer) ($m^2/g$): 220 10 $SiO_2$ 1 $H_2O$, 2% soluble salts
SAXS ($m^2/g$): 123.3( ± 4.9)
BET ($m^2/g$): 189.16
impurity/coating : 10 $SiO_2$ 1 $H_2O$, 2% soluble salts

## Results and discussions

### Specific surface area

**Mean** 189.16 m²/g

**Standard deviation**

**Remarks on results including tables and figures**

total pore volume (ml/g): 0.7905 microsurface area ($m^2$/g): 30.044 micropore volume (ml/g): 0.01181

## Overall remarks, attachments

### Overall remarks

Isotherms of nitrogen sorption experiments at 77 K for NM-200 is shown in the attached file.

## Attached background material

**Attached document** BET-NM-200.doc / 41.5 KB (application/msword): ENV/JM/MONO(2015)14/ANN4

**Remarks**

### 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

# *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

### Data access

other: owner: NANOGENOTOX

## 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.

## Details on methods and data evaluation

1) Sample preparation:NM-200 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 3600 s, leading to transmissions of about 0.25. USAXS measurements were performed in 1 mm or 1.5 mm non-sticky double kapton cells
3) 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) 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-200

**State of test material**

other: fluffy powder

**Results and discussions**

### Specific surface area

**Mean** 123 $m^2/g$

**Standard** 4.9 $m^2/g$

**deviation**

### Remarks on results including tables and figures

The figure with the SAXS and USAXS curves is shown in the attached file: SAXS and USAXS for NM-200

### Overall remarks, attachments

### Attached background material

**Attached document** SAXS and USAXS for NM-200.doc / 157.5 KB (application/msword): ENV/JM/MONO(2015)14/ANN4

**Remarks**

### 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

## *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.The results from both analytical methods show a big dferences for NM-200 material.BET specific surface area ($m^2/g$): 189.16SAXS specific surface area ($m^2/g$): 123.3(4.9). Assessed from the methodology, most of the differences may be explained by the combined errors indensity and placement of plateau. Other explanations may come from the difference in thermal treatment and outgassing of the powders before BET analysis. Indeed, thermogravimetric

analysis showed a loss 2 wt % in the analysis of NM-200, which could come from organic coating or water, “wrapping” the nanoparticles and therefore responsible for a decrease of the X-ray contrast and subsequently of the specific surface area seen by SAXS.It should also be mentioned that the Porod plateau is determined in a q range up to 0.3 Å-1, which corresponds in the direct space to dimensions down to 2 nm. This means that it is very difficult to estimate a roughness smaller than 2 nm in these conditions (leading to an additional surface area).This could explain why, in BET measurements, $N_2$ molecules, smaller than 2 nm, might “see” more surface in general.

**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 NANOGENOTOXdata. This suggests that producer instrumental capacity and the SOPs for making BET analysis aresimilar or of same quality as the procedures used in NANOGENOTOX. All being well as SAXS dataconfirms the obtained results.

## 4.29 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

### *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** | | **Report no.** | |
| **Owner company** | NANOGENOTOX | | |
| **Company study no.** | | **Report date** | |

**Data access**

other: data owner: NANOGENOTOX

## 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:

o 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)o 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" solution

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 $NO_3^-$ /$OH^-$

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 $SiO_2$ 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 $SiO_2$ by CEA.doc / 330 KB (application/msword) : SIAR |
| **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

o Equilibrium pH of the suspensions are measured and considered as pH values for the reported results.
o 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.

o 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.
o 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. o The parameters used for dispersant and material properties are available in the attached file with the SOP for Zetametry.
o 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.
o Three measurements are performed and averaged for reporting. o For unstable samples, measurements are performed on supernatants.
o Zeta potentials are then plotted against pH to determine the stability domains and isoelectric points (IEP)-see attached file with the figure.

### Reproducibility

o Three measurements are performed and averaged for reporting.
o For unstable samples, measurements are performed on supernatants.
o 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-200

### 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-200 form stable suspension, with negatively to neutral chargednanoparticles. The zeta potential, however, varied greatly as function of pH and reached -45 mV around pH 7.

### Overall remarks, attachments

### Attached background material

**Attached document** zeta potential as a function of pH CEA $SiO_2$.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): ENV/JM/MONO(2015)14/ANN5

### 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-200 form stable suspension, with negatively to neutral charged nanoparticles. The zeta potential, however, varied greatly as function of pH and reached -45 mV around pH 7.

## 4.30 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 IR Spectroscopy

**Rationale for reliability** Precipitated synthetic amorphous silica provided to the sponsoring program is hydrophilic silicon dioxide which have a higher surface energy (solid) than the surface tension of water, which is 72 mN/m (same dimension than 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 characterised: isolated, vicinal and geminal silanols. (See IR Spectroscopy).

### Data gathering

#### Test materials
#### Test material equivalent to submission substance identity

yes

# *Endpoint study record: Surface chemistry by EDS by IMC-BAS*

## 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** | Keld Alstrup 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

### Principles of method if other than guideline

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

### Details on methods and data evaluation

elements were reported as semi-quantitative results

#### *Used Protocols*

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.

### Data gathering

**Test materials**
**Reference Material/Nanomaterial and Sample identification number**

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

**State of test material**

other: powder

### 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**

The following elements were identified in the surface: O (71.43 at%), Si (20.30 at%), C (5.96 at%) and Na (1.83 at%). The presence of C is considered to be due to surface contamination.

### Cross-reference to other study

http://www.nanogenotox.eu/

## 4.31 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

### Data access

other: owner: NANOGENOTOX

### 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**

o 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).

o 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.

o 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.

o 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).

o 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.o 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.

o The saturation test was performed using 2 grams of powder and rotation for 60 seconds.

o Then the actual triplicate tests were completed using 6 grams of test material per run.

o After each run the drum was emptied by pouring out the residual powder and gently tapping the drum three times with a rubber hammer.

o 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.

o 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.

o 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.

o 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.

o 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-200

**State of test material**

other: fluffy powder

### Results and discussions

### Remarks on results including tables and figures

Test mass (g): Dustiness indexNumber (1/mg) CPC: 6.16 E+06

Inhalable (Mass (mg/kg)): 6459 (± 273) Respirable (Mass (mg/kg)): 293 (± 193)

### Overall remarks, attachments

### Overall remarks

The NM-200 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

## *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

### Data access

other: Owner: NANOGENOTOX

## Materials and methods

### Details on methods and data evaluation

Vortex Shaker (VS) method: The 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 vial together 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 have 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 thedata from the ELPI: spherical particle with a density equal to the density of the condensed phase of thematerial 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 0.8 μm). 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 original protocol 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 therespirable 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 weighingprocedure 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 expressed in 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 weighingprocedure 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 concentrationssubstrates used PVC GELMAN GLA-5000 5µm / 25 mmDifferent 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-200

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

## Results and discussions

### Remarks on results including tables and figures

Gravimetric water content and bulk density Sample mass (mg) 121
Water content (wt % dry) 8%
Bulk density (g/cm$^3$) 0.12
Number-based and mass-based dustiness indexes of NM-200 Test mass (mg): 60.2
Dustiness index Time = 180s
Number (1/g)CPC (S.D): 2.9E+06 (2.9E+05)ELPIa (S.D): 1.1E+07 (6.0E+06)
Time=3600s CPC (S.D): 8.2E+06 (1.3E+06) Respirable (S.D): 3.4E+04 (3.04E-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 beenDeveloped 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 thegenerated 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 existingliterature.- 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 rotatingdrum method.Dustiness as quantified by particle number or by mass-based dustiness index had for both methods a largerange. These findings suggest a corresponding large difference in exposure potential. It is however difficult tosay 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 thetwo 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 particles released from handling.

# 4.32 Porosity

## *Endpoint study record: Porosity.001*

### Administrative Data

**Purpose flag** weight of evidence ( ) 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-200 (red line)

### Attached background material

**Attached document** NM-200 Por.pdf / 21.91 KB (application/pdf): SIAR

**Remarks**

**Attached document** NM-200 Por 2.pdf / 90.32 KB (application/pdf): SIAR

**Remarks**

## *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

### 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, formationand potential applications of different natural materials. For this reason it is important to determineand control them accurately. The most widely used technique for estimating surface area is the socalled BET method (Brünauer, Emmett and Teller, 19389. 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 inlayers 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 thea dsorbate (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 theequilibrium 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-200

**Test material identity**

**Identifier** CAS number

**Identity** 7631-86-9

**State of test material**

other: fluffy powder

**Results and discussions**

**Porosity (fraction of void space in the material)**

**Mean** 0.79

**Standard deviation**

**Remarks on results including tables and figures**

total pore volume (ml/g): 0.7905micropore volume (ml/g): 0.01181

## 4.35 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 characteristics 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 characteristics is inherent to the substance and is not linked to a specific lot.

# 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** | 09 Dec. 1991 - 13 Dec. 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-91-0059-02 |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 92-0141-DGO | **Report date** | 1992-09-16 |

### 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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

### Details on test material
$SiO_2$: (approx. 98 % $SiO_2$): CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8

### Confidential details on test material
The test substance is equivalent to NM-200.

### 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- 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): 2.1 +-0.1 cm- Weight at study initiation (mean and range, SD): 0.07 +-0.01 g- Food during test: no

## 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 $CaCO_3$

***Test temperature***

24.2 - 25.3

***pH***

7.3 - 8.3

***Dissolved oxygen***

7.3 - 8.5 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, 1500 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. 0.5 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, morphologial 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

### *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 applicable

***Reported statistics and error estimates***

not applicable

### Applicant's summary and conclusion

### Validity criteria fulfilled

yes

### Conclusions

The 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** | 12 Dec. 1991 - 13 Dec. 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-91-0059-01 |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 92-0162-DGO | **Report date** | 1992-11-20 |

### 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 oxgen content was 4.2 mg/L after 24 h, 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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

## Details on test material

$SiO_2$: (approx. 98 % $SiO_2$): CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8

## Confidential details on test material

The test substance is equivalent to NM-200.

## 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, and standing for 4 h before use
- Eluate: no, suspensions used after settling
- Differential loading: yes, limit tests with 1000 and 10,000 mg/L
-Evidence of undissolved material: The test media remained turbid throughout the test, and starchy particles were observed on the bottom of the test vessels
Controls: Synthetic medium from groundwater

## 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)
Every week, new cultures started with approx. 125 daphniae of the same age (1 day old), fed on Chlorella (about 4 x10^9 cells and about 0.13 g yeast per 4 L-
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 CaCO3

***Test temperature***

20 ±1°C

***pH***

7.4 - 8.1

***Dissolved oxygen***

4.2 - 8.4 mg/L

***Salinity***

not applicable

***Nominal and measured concentrations***

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

***Details on test conditions***

TEST SYSTEM
- Test vessel: 150-mL beaker
- Type: open - Material, size, headspace, fill volume: glass beaker, 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 (with synthetic medium)
- 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: 1.7 mg/L
- Alkalinity: no data
- Ca/Mg ratio: 1.83
- 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.** | > 10000 mg/L | | |
| **Nominal/Measured** | nominal | | |
| **Conc. based on** | test mat. | **Basis for effect** | mobility : 1/40 animals were immobile = 2.5 % |
| **Remarks (e.g. 95% CL)** | | | |

### *Details on results*

-- Mortality/immobility of control: 0/40- Other adverse effects control: none
- Abnormal responses: Immobilisation effects can be attributed to physical hampering of the daphnias.
- Detailed results (see also Table under "Remarks on results..." below): 3/40 (15 %) and 1/40 (2.5 %) of the daphniae were immobile/dead at a loading of 1000 and 10000 mg/L, respectively (non-filtered suspension after settling).

### *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.

## Overall remarks, attachments

### Attached background material

**Attached document** Pic 1 NM-201.jpg / 45.07 KB (image/jpeg): ENV/JM/MONO(2015)14/ANN9

**Remarks**

## Applicant's summary and conclusion

### Conclusions

The observed effects were not dose related, and it is likely that they are caused by physical hampering of the test animals.

# 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

## 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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

## Radiolabelling

no

## Test materials

### Details on test material

- Test material: $SiO_2$ CAS-Name: Silica, precipitated, crystalline-free; CAS-No.: 112926-00-8
Surface area (Ströhlein): 160 - 195 $m^2/g$
Primary particle size: see Test Condition
- Substance type: inorganic
- Physical state: solid
- Surface area (BET): 192 $m^2/g$ (Report p. 64 Specification Certificate)
- Analytical purity: >98 % ($SiO_2$)

- Impurites: 0.8 % $Na_2O$, 0.2 $Al_2O_3$
- 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

## Confidential details on test material

The test substance is equivalent to NM-200.

## 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

## 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

35 mg/$m^3$ (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: ca. 7 wks (males) (from lung, see Table below)

**Test No.** #2 Half-life 2nd: ca. 7 wks (males) (from lung, see Table below)

### Metabolite characterisation studies

### Metabolites identified

not measured

### Remarks on results including tables and figures

see attached background material

## Overall remarks, attachments

### Overall remarks

SILICA DEPOSITION Silica could be detected in lungs of all exposed rats at the end of the exposure period: In all males, residual amounts were still present after half a year post-exposure, while only one female rat showed Si in the lung at that time. After exposure (one week post-exposure), in 3/10 males and 5/10 females Si was found in the lymph nodes, which slowly declined during recovery.

### Attached background material

**Attached document** NM-201 Table 1.pdf / 38.79 KB (application/pdf): ENV/JM/MONO(2015)14/ANN10

**Remarks**

## Applicant's summary and conclusion

### Interpretation of results

no bioaccumulation potential based on study results

## *Endpoint study record: Basic toxicokinetics_NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### 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_NM-200_Gavage_ISS.docx / 17.73 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 NM-200 following repeated oral administration of 20 mg/kg

#### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: Basic toxicokinetics_NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

## 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_NM-200_IV_ISS.docx / 21.94 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

## Applicant's summary and conclusion

### Interpretation of results

other: Single administration: high concentrations of Si were detected in liver >spleen, lungs. Decrease in Si level at or below LOQ in all organs at 90 days. Repeated administrations: At days 6, Si concentrations very high in liver> spleen and lungs.

### Conclusions

Bioaccumulation of Si in liver >spleen, lungs at day 2 and 6 which decreases at or below the limit of quantification at day 90 following single dose whereas it is still above the control in liver and spleen following repeated administrations.

### 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** Oct. 1985

**Reliability** 1 (reliable without restriction)

**Rationale for reliability** GLP guideline study

#### Data source

#### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Guillot JP, Braise J | **Year** | 1986 |
| **Title** | Essai de toxicité par voie orale chez le rat | | |
| **Bibliographic source** | unpublished report | | |
| **Testing laboratory** | Hazelton - IFT, France | **Report no.** | 601203 |
| **Owner company** | Rhone-Poulenc | | |
| **Company study no.** | | **Report date** | 1986-01-07 |

#### Data access

other: data owner or letter of access

#### Data protection claimed

yes, but willing to share

#### Materials and methods

#### Test type

acute toxic class method

**Test guideline**

**Qualifier** according to

**Guideline** OECD Guideline 401 (Acute Oral Toxicity)

**Deviations**

#### 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-200.

### Details on test material

CAS-Name: Silica, precipitated, cryst.-free, CAS No. 112926-00-8 / pH 7.75, sulfates 0.4 %, BET surface: 255 $m^2/g$

### Confidential details on test material

The test substance is equivalent to NM-200.

## Test animals

### Species

rat

### Strain

Sprague-Dawley

### Sex

male/female

### *Details on test animals and environmental conditions*

TEST ANIMALS- Source: IFFA CREDO, saint Germain sur l´Arbresle
- Age at study initiation: 6 - 7 weeks
- Weight at study initiation: 160 - 200 g (male), 140 - 180 (female)
- Fasting period before study: 16 - 20 before start
- Housing: 5/cage (stainless steel)
- Water: ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ±3
- Humidity (%): 30 - 70 %
- Air changes (per hr): 10x

## Administration / exposure

### Route of administration

oral: gavage

### Vehicle

other: dispersion of 10 % gum arabicum in water

***Details on oral exposure***

VEHICLE
- Concentration in vehicle: 500 g/L
- Amount of vehicle: 10 mL/kg (in untreated control)
- Justification for choice of vehicle: suspending the test material and stabilising the suspension

MAXIMUM DOSE VOLUME APPLIED:
total dosage volume including TS: 2, 5, and 10 mL/kg bw at 1000, 2500, and 5000 mg/kg, respectively

### Doses

1000, 2500, and 5000 mg/kg (pre-study); 5000 mg/kg (main study)

### 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: --
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight

***Statistics***

not relevant

## Results and discussions

### Effect levels

| | |
|---|---|
| **Sex** | male/female |
| **Endpoint** | LD50 |
| **Effect level** | > 5000 mg/kg bw |
| **95% CL** | |
| **Remarks** | |

***Mortality***

none

***Clinical signs***

no particular findings

***Body weight***

normal

***Gross pathology***

no particular findings

## 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** | Nov. 1977 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability** | Comparable to guideline study with acceptable restrictions | | |

## Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Leuschner F | **Year** | 1977 |
| **Title** | Pruefung der akuten Toxizität von Testsubstanz 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 Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 77-0016-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** 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-200.

### Details on test material

$SiO_2$: 97-98 % ($SiO_2$): CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8

### Confidential details on test material

The test substance is equivalent to NM-200.

**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

## 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

MAXIMUM DOSE VOLUME APPLIED: yes

## Doses

2000 and 5000 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** > 5000 mg/kg bw
**95% CL**
**Remarks**

***Mortality***
no mortality

***Clinical signs***
no particular findings

***Body weight***
no particular findings

***Gross pathology***
no particular findings

### 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** 02 Feb. - 16 Feb. 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 83.111/221216 |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 83-0062-DGT | **Report date** | 1983-05-20 |

### 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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

## Details on test material

$SiO_2$ >98 % ($SiO_2$): CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8 Surface area (Ströhlein): 160 - 195 $m^2$/gPrimary particle size: see Test Condition

## Confidential details on test material

The test substance is equivalent to NM-200.

## 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:
- Weight at study initiation: 168 - 179 g: av. 174 g (male); 142 - 146 g: av. 144 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 $m^3$/hour ==> 0.8/h
- 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
- Treatment of exhaust air: no data
- Temperature, humidity, pressure in air chamber: no data

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
- Samples taken from breathing zone: no data

TEST ATMOSPHERE
- Particle size distribution: approx. 65 mass % ≤ 6 µm (note: 45 mass % with < 5 µm are stated on p.6 of the report. This is not in agreement with the profile given in Table 1., see below.) from Report Tab. 1:

| Distribution aerodynamic in % of total weight) | aerodynamic diameter |
|---|---|
| 1.8 | 0.47 |
| 2.8 | 0.7 |
| 4.3 | 1.1 |
| 5.0 | 1.7 |
| 8.1 | 2.5 |
| 9.4 | 3.4 |
| 14.3 | 4.3 |
| 19.2 | 5.7 |
| 35.1 | ≥7.7 |

MMAD (Mass median aerodynamic diameter) / GSD (Geometric st. dev.):
MMAD - = ~0.6 µm
GSD: no data
Note: calculated from the VMD (Volume Mean Diameter) of 11.5 µm multiplied with the density of about 0.05 g/$cm^3$.

CLASS METHOD (if applicable)
- Rationale for the selection of the starting concentration: maximum attainable concentration

**Duration of exposure**

4 h **Remarks**

**Concentrations**

maximum attainable concentration: 691 mg/$m^3$ (range: 650 - 725 mg/$m^3$)Nominal concentration: 36.7 g/$m^3$

## No. of animals per sex per dose

5

***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 technical maximum attainable aerosol concentration in the chamber ranged from 650 to 725 mg/m$^3$, while the nominal concentration was 36.7 g/m$^3$. About 65 % of the aerosol comprised particles with an aerodynamic diameter of <6 µm (part of respirable fraction) (note: Summation of the fractions of <5.7 µm in Table 1 results in 65 %, not 45 % as indicated in the report.)[see above: "Details on inhalation exposure"].

## Results and discussions

### Effect levels

| | |
|---|---|
| **Sex** | male/female |
| **Endpoint** | LC0 |
| **Effect level** | ≥ 0.69 mg/L air (analytical) |
| **95% CL** | |
| **Exp. duration** | 4 h |
| **Remarks** | |
| **Sex** | male/female |
| **Endpoint** | LC50 |
| **Effect level** | ≥ 0.69 mg/L air (analytical) |
| **95% CL** | |
| **Exp. duration** | 4 h |
| **Remarks** | |

### *Mortality*

none

### *Clinical signs*

Restlessness, half-closed eyes

### *Body weight*

males: normal; females: some delay until day 2, then normal

***Gross pathology***

no particular findings

***Other findings***

none

**Remarks on results including tables and figures**

No clinical symptoms except some restlessness and eye closing. Body weight gain was not affected in males, but females hardly gained weight during two days after exposure, however, subsequently, showed normal development. No findings at autopsy after 14 d post-treatment.

**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** | 07 - 10 July 1992 |
| **Reliability** | 1 (reliable without restriction) | | |
| **Rationale for reliability** | Test procedure according to national standards, result evaluable in terms of today´s criteria | | |

### Data source

**Reference**

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Mercier O | **Year** | 1992 |
| **Title** | Test pour la determination de l´indice de iritation primaire cutanée chez le lapin | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | Hazelton France | **Report no.** | 207372 |
| **Owner company** | Rhone-Poulenc (Rhodia) | | |
| **Company study no.** | | **Report date** | 1992-10-19 |

### 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: National standard protocol (No. IPC/05-92) corresponding to US EPA

**Deviations**

### Principles of method if other than guideline

24 h exposure on intact and abraded skin

### GLP compliance

yes

### 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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

### Details on test material

CAS name: Silica, precipitated, CAS No. 112926-00-8

### Confidential details on test material

The test substance is equivalent to NM-200.

## Test animals

### Species

rabbit

### Strain

New Zealand White

## Test system

### Type of coverage

occlusive

### Preparation of test site

other: intact and abraded

### Vehicle

water

### *Amount/concentration applied*

Concentration: 190 mgVolume: 0.5 ml

### Duration of treatment / exposure

24 hour(s)

### Observation period

3 days reading on 24 and 72 hour after administration of the test material

### Number of animals

6

### Control animals

no

### *Details on study design*

The substance was applied as aqueous suspension (17 % w/w = approx. 0.38 g/ml), 0.5 ml = 190 mg onto the intact and scarified skin.

## Results and discussions

### Irritation / corrosion results

**Irritation parameter** primary dermal irritation index (PDII)

**Basis**

| | |
|---|---|
| **Time point** | 24 h |
| **Score** | 0.29 |
| **Max. score** | 8 |
| **Reversibility** | fully reversible |
| **Remarks** | No effect at 72 h |

***Irritant/corrosive response data***

Slight erythemas were seen in 4/6 animals 0.5 h after 24-h exposure. No signs of irritation after 72 h.

### Applicant's summary and conclusion

### Interpretation of results

not irritating

### Criteria used for interpretation of results

EU

### Conclusions

No classification (EU and GHS)

## *Endpoint study record: Skin irritation / corrosion.002*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting 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 von Testsubstanz an der Kaninchenhaut (Patch-Test) | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | Laboratorium für Pharmakologie und Toxikologie (LPT), Hamburg | **Report no.** | |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 78-0010-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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

### Details on test material

$SiO_2$: >98 % ($SiO_2$): CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8 Surface area (Ströhlein): 160 - 195 $m^2$/g silica

### Confidential details on test material

The test substance is equivalent to NM-200.

### 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
- 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 - % coverage:
- Type of wrap if used: plasic foil

REMOVAL OF TEST SUBSTANCE
- Washing (if done): no data
- 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 von Testsubstanz bei einmaliger Applikation | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | Laboratorium für Pharmakologie und Toxikologie (LPT), Hamburg | **Report no.** | |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 78-0011-DKT | **Report date** | 1978-06-20 |

### 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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

### Details on test material

$SiO_2$ >98 % ($SiO_2$): CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8
Surface area (Ströhlein): 160 - 195 $m^2/g$

### Confidential details on test material

The test substance is equivalent to NM-200.

## 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

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21±2 °C
- Humidity (%): 60 ± 3
- 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

### *Endpoint study record: Repeated dose toxicity: oral.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) |
| **Rationale for reliability** | GLP guideline study |

**Data source**

**Reference**

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Lewin G | **Year** | 2011 |
| **Title** | 28-Day Oral Toxicity Study of Synthetic Amorphous Silica in Wistar (WU) Rats | | |
| **Bibliographic source** | | | |
| **Testing laboratory** | Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany | **Report no.** | 02G10031 |
| **Owner company** | CEFIC Brussels/Belgium | | |
| **Company study no.** | | **Report date** | 2011-10-13 |

**Data access**

other: data owner or letter of access

**Data protection claimed**

yes, but willing to share

## Materials and methods

**Test type**

subacute

### Limit test

no

### Test guideline

**Qualifier** equivalent or similar to

**Guideline** OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)

**Deviations** yes only males

### 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

- Name of test material (as cited in study report): NM-200 (Synthetic amorphous silica)
- CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8
- Substance type: inorganic
- Physical state: solid, white powder
- Analytical purity: 96.5 % ($SiO_2$)
- Ignition loss: 8.9 %
- Specific surface (BET): BET 230 $m^2/g$
- Lot/batch No.: Master batch
- Stability under test conditions: stable
- Storage condition of test material: Ambient temperature, dry place, closed container, in the dark

## Test animals

### Species

rat

### Strain

Wistar

### Sex

male

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

TEST ANIMALS
- Source: Charles River Deutschland, Sulzfeld, Germany
- Age at delivery: approx. 5 wks
- Weight at study initiation: individual 218 - 223 g (Report, Table 3)
- Fasting period before study: no-
Housing: 5 animals per cage (Makrolon® Type IV cages)
- Diet: ad libitum
- Water: ad libitum- Acclimation period: 14 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ± 2 °C
- Humidity (%): 30 - 70 %
- Photoperiod (hrs dark / hrs light): 12 / 12

## Administration / exposure

### Route of administration

oral: gavage

### Vehicle

other: Methylhydroxypropylcellulose

***Details on oral exposure***

PREPARATION OF DOSING SOLUTIONS:VEHICLE
- Justification for use and choice of vehicle: non-toxic and well tolerable, stablising homogeneity of the silica dispersion
- Concentration in vehicle: 0.5 % (w/v) in deionised water
- Amount of vehicle (if gavage): 7.5 mL/kg bw/d (including test substance)
- Frequency of preparation: no data, probably daily
- Storage: no data

TEST SUBSTANCE SOLUTION
- Frequency of preparation: daily
- A suspension of Synthetic Amorphous Silica in 0.5 % methylhydroxypropylcellulose was prepared by adding the vehicle ad 30 ml to a pre-weighed amount (0.40, 1.20, 4.00, or 4.00 g) of Synthetic

Amorphous Silica for group 2, 3, 4, and 6, respectively.

**Analytical verification of doses or concentrations**

no

**Duration of treatment / exposure**

28 days

**Frequency of treatment**

1x/day

**Doses/concentrations**

100, 300, and 1000 mg/kg bw/d; recovery group: 1000 mg/kg bw/d

**Basis** other: nominal daily dose (in 7.5 mL/kg bw/d)

**No. of animals per sex per dose**

5 males

**Control animals**

yes, concurrent vehicle

***Details on study design***

- Dose selection rationale: based on 14-d range-finding study
- Post-exposure recovery period in satellite groups: 14 days

***Positive control***

none

**Examinations**

***Observations and examinations performed and frequency***

CAGE SIDE OBSERVATIONS:
Yes- Time schedule: 1x/d
- Cage side observations checked in table 2 were included.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: 1x/d

BODY WEIGHT: Yes
- Time schedule for examinations: weekly

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/animal/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: No

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

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

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: before sacrifice
- Anaesthetic used for blood collection: no
– Animals fasted: Yes
- How many animals: all
- Parameters checked in tables 9 - 11 were examined.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: before sacrifice
- Animals fasted: Yes - How many animals: all
- Parameters checked in table 12 - 13 were examined.

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: Yes
- Time schedule for examinations: last week of treatment- Dose groups that were examined: control (group 1) and hoghest dose (group 4)
- Battery of functions tested: sensory activity (pupil reflex, righting reflex) / grip strength / motor activity

### *Sacrifice and pathology*

GROSS PATHOLOGY: Yes (see Appendix K)
HISTOPATHOLOGY: Yes (see table 18). Complete histopathological examination conducted in all animals of groups 1 (control) and 4 (high dose).

### *Other examinations*

- Determination of silicon concentration in liver, kidney, and blood by ICP-MS (Report 5.9.1 and Table 19: see below "Any other information on results").
- Determination of silicon particles in liver, kidney, and mesenterial lymph nodes using electron microscopy.Transmission electron microscopy (TEM) was used to spot check within the liver, kidney and mesenteric lymph nodes. (see Report 5.9.2, and Table 20)

#### *Statistics*

Differences between groups were considered case by case as statistically significant for $p<0.05$. Data were analyzed using analysis of variance. If the group means differed significantly according to the analysis of variance, the means of the treatment groups were compared with the means of the control group, using DUNNETT's modification of the t-test.Kruskal-Wallis ANOVA and Mann-Whitney U-test were applied in the case of non-homogeneous data. The statistical evaluation of the histopathological findings was done with the two-tailed FISHER test using the PROVANTIS system. For comparisons of semiquantitative data, the Chi-square test was used.

## Results and discussions

### Effect levels

| | |
|---|---|
| **Endpoint** | NOEL based on test material |
| **Effect level** | 1000 mg/kg bw/day (actual dose received) |
| **Sex** | male |
| **Basis for effect level / Remarks** | overall effects |

### Observations

***Clinical signs and mortality***

no effects

***Body weight and weight gain***

no effects

***Food consumption and compound intake (if feeding study)***

no effects

***Food efficiency***

not examined

***Water consumption and compound intake (if drinking water study)***

no data

***Ophthalmoscopic examination***

not examined

***Haematology***

no effects

***Clinical chemistry***

no effects

***Urinalysis***

not examined

***Neurobehaviour***

no effects

***Organ weights***

no effects

***Gross pathology***

no effects

***Histopathology: non-neoplastic***

no effects

***Histopathology: neoplastic***

no effects

***Details on results***

No particular findings

**Remarks on results including tables and figures**

see attachment

## Overall remarks, attachments

## Attached background material

**Attached document** FhG Item 2011 7.5.pdf / 67.03 KB (application/pdf): ENV/JM/MONO(2015)14/ANN10

**Remarks**

## Applicant's summary and conclusion

## Conclusions

No toxicologically significant adverse effects were observed after oral administration of synthetic amorphous silica to male rats for 28 days. The NOEL is 1000 mg/kg bw/day, the highest dose tested.

## Executive summary

The objective of this study was to evaluate the possible toxicity of Synthetic Amorphous Silica after oral administration (gavage) in rats for 28 days and an additional 14-day recovery period, based on the OECD Guideline 407, but using only one sex.100, 300 and 1000 mg/kg bw (body weight) Synthetic Amorphous Silica were used as dose levels in this study. The study consisted of 5 male rats per group. Rats were treated daily either with the vehicle (0.5% methylhydroxypropylcellulose, control groups) or with Synthetic Amorphous Silica suspended in the vehicle for 28 consecutive days. The rats in groups 5 (control recovery) and 6 (high dose recovery) were kept for additional 14 days without treatment.In animals of the control and top-dose group (1 and 4), silicon ion concentrations were determined in kidney, liver and blood by chemical analysis. Furthermore, determination of particles resembling silica particles was performed by transmission electron microscopy in mesenteric lymph nodes, kidney and liver.No death occurred during the study and no adverse clinical symptoms were observed. No effects on food consumption or body weight were seen. The measurements of the spontaneous locomotor activity and the functional observational battery displayed no influence by the treatment. Evaluation of haematological and clinical chemistry parameters did not reveal any treatment related effects. Decreases of the partial thromboplastin time (PTT), white blood cell count (WBC) and lymphocyte count (LYMC) as well as cholinesterase (CHE) and glucose (GLUC) in group 3 (mid dose) after 28 d of exposure were considered not treatment-related. Creatinin kinase (CK) and blood urea (UREA) concentration were

mildly decreased in group 6 (high dose recovery) after a two week recovery period. All values were within the normal range, and the changes were considered not treatment-related and to be due to inter individual variability.During necropsy, no substance-related findings were observed. No effects were seen on organ weights or the organ weight to bodyweight ratio. During histopathological examination, no substance-related findings were observed in the examined organs of males of the control and high dose group.Toxicological analysis of silica ion concentration (non-GLP) in blood, kidney and liver tissue did not reveal differences between the control and the high dose group. This result is most likely due to the naturally occurring high background values of silica. Transmission electron microscopy (non-GLP) found electron dense structures composed of irregular homogenous to fine granular material in the cytoplasm of mesenteric lymph nodes cells, liver cells and kidney cells of all animals from the control and from the high dose group. The granular structures measured only few nanometer. However, these structures did not have the shape or appearance of amorphous material such as amorphous silica.

## *Endpoint study record: Repeated dose toxicity: oral.002*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study (X) robust study summary ( ) used for classification ( ) used for MSDS | | |
| **Study result type** | experimental result | **Study period** | 27 Oct. 1980 - 27 Jan. 1981 |
| **Reliability** | 1 (reliable without restriction) | | |
| **Rationale for reliability** | Comparable to guideline study, well documented. | | |

### Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Til HP, Hollanders MH, Beems RB | **Year** | 1981 |
| **Title** | Subchronic (13 week) oral toxicity study in rats | | |
| **Bibliographic source** | Unpublished Report | | |
| **Testing laboratory** | TNO Division for Nutrition and Food Research, Zeist/NL | **Report no.** | V81.268/201741 |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG US-IT-No. 81-0016-DKT | **Report date** | 1981-08-01 |

### Data access

other: data owner or letter of access

### Data protection claimed

yes, but willing to share

### Materials and methods

### Test type

subchronic

### Test guideline

**Qualifier** equivalent or similar to

**Guideline** OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)

**Deviations**

### 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-200.

### Details on test material

97-98 % ($SiO_2$): CAS-Name: Silica, precipitated,cryst.-free; CAS-No.: 112926-00-8

### Confidential details on test material

The test substance is equivalent to NM-200.

### Test animals

### Species

rat

### Strain

Wistar

### Sex

male/female

## Administration / exposure

### Route of administration

oral: feed

***Details on oral exposure***

DIET PREPARATION
Treated feed: 6-kg batches mixed with the test material for 2 min, freshly prepared 5x/13 weeks and stored at 15 °C until use

### Analytical verification of doses or concentrations

yes

***Details on analytical verification of doses or concentrations***

Mean effective (analytical) silica levels in the diet were about 0.4-0.7, 1.7-1.9, 6.5-7.0 % (Tab. 1, p. 17). These dietary levels resulted in indicated doses of test substance, based on specified mean food intake and body weights

### Duration of treatment / exposure

13 weeks

### Frequency of treatment

daily, continuous

### Doses/concentrations

approx. 0.5, 2 and 6.7 % Si

**Basis** other: based on Si analysis in the diet (i.e. Si concentration)
Mean effective (analytical) silica levels in the diet were about 0.4-0.7, 1.7-1.9, 6.5-7.0 % (Tab. 1, p. 17). These dietary levels result in indicated doses of test substance, based on specified mean estimated doses: 300-330, 1200-1400, 4000-4500 mg Substance/(kg*d)

**Basis** nominal in diet

### No. of animals per sex per dose

10 (5 per sex and cage)

### Control animals

yes, concurrent no treatment

***Details on study design***

Post-exposure period: no

### Examinations

***Observations and examinations performed and frequency***

CAGE SIDE OBSERVATIONS: Yes

DETAILED CLINICAL OBSERVATIONS: Yes

BODY WEIGHT: Yes

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: 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

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes

CLINICAL CHEMISTRY: Yes

URINALYSIS: Yes

NEUROBEHAVIOURAL EXAMINATION: No

## Results and discussions

### Effect levels

| | |
|---|---|
| **Endpoint** | NOEL |
| **Effect level** | 6.7 % in feed |
| **Sex** | male/female |
| **Basis for effect level / Remarks** | overall effectsclinical signs; mortality; body weight; food consumption; food efficiency; water consumption and compound intake; haematology; clinical chemistry; urinalysis; gross pathology; organ weights; histopathology |
| **Endpoint** | NOEL highest dose |
| **Effect level** | ca. 4000 —≤ 4500 mg/kg bw/day (nominal) |
| **Sex** | male/female |
| **Basis for effect level / Remarks** | see above |

### Observations

***Clinical signs and mortality***

no effects

***Body weight and weight gain***

no effects

***Food consumption and compound intake (if feeding study)***

yes

***Food efficiency***

no effects

***Water consumption and compound intake (if drinking water study)***

no effects

***Ophthalmoscopic examination***

not examined

***Haematology***

no effects

***Clinical chemistry***

no effects

***Urinalysis***

no effects

***Neurobehaviour***

no effects

***Organ weights***

no effects

***Gross pathology***

no effects

***Histopathology: non-neoplastic***

no effects

***Histopathology: neoplastic***

not examined

***Details on results***

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study)
Mean food intake was slightly increased in the female top-dose group (some +5 % after 4 wks) with no corresponding body-weight gain, but barely seen in males (Tab. 5, p. 23).

FOOD EFFICIENCY

In females (high dose): The apparently reduced food efficiency may be due to the rather high amount of inert test substance.

### Remarks on results including tables and figures

No clinical symptoms or other findings including haematological, blood-chemical and urinary parameters. Mean food intake was slightly increased in the female top-dose group (some +5 % after 4 wks) with no corresponding body-weight gain, but barely seen in males (Tab. 5, p. 23). The reduced food efficiency may be due to the rather high amount of inert test substance. Water consumption was normal throughout. Gross and microscopical examinations did not reveal any (histo-) pathological changes that could be attributed to the feeding of the test substance.

## 7.5.2 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: 1 |
| **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 of test substance 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 Degussa GmbH | | |
| **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, but willing to share

## Materials and methods

### Test type

subchronic

### Limit test

yes

### 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 lymph nodes. Post-exposure recovery period up to one year. One high exposure level only within a combined study (in contrast to NM-203, see other entry).

### Principles of method if other than guideline

Comparative study including test substance and other Silicas 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

**Identifier** other:

**Identity** The test substance is equivalent to NM-200.

### Details on test material

- Test material: $SiO_2$ similar to NM-201 CAS-Name: Silica, precipitated, crystalline-free; CAS-No.: 112926-00-8
- Surface area (Ströhlein): 160 - 195 $m^2/g$
- Primary particle size: see Test Condition
- Substance type: inorganic- Physical state: solid
- Surface area (BET): 192 $m^2/g$ (Report p. 64 Specification Certificate)
- Analytical purity: >98 % ($SiO_2$)
- Impurites: 0.8 % $Na_2O$, 0.2 $Al_2O_3$
- Stability under test conditions: stable- Storage condition of test material: room temperature

### Confidential details on test material

The test substance is equivalent to NM-200.

## 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 time 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 2: Daily mean concentrations are documented:based on 254 measurements: 34.91 (SEM 0.49) mg/$m^3$

### Duration of treatment / exposure

13 weeks

### Frequency of treatment

6 hours/day, 5 days/week

### Doses/concentrations

35 mg/$m^3$ (mean analytical values)

**Basis** analytical conc.

30 mg/m3 (target concentration)

**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 Similar NM-201: assigned dose groups F, 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/$m^3$) 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
- Report 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-

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

- Report 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 Report 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 (Report Table 63 - 67)
Relative organ weights (Report Table 37 - 56)

HISTOPATHOLOGY: Yes (Report Table 68 - 73), in particular lung and lymph nodesin addition:Si contents of lung and lymph nodes (Report Tables 59 - 62)
Collagen content in lung (Report Tables 57/58)

### *Other examinations*

Relative organ weights (Table 37 - 56)

#### *Statistics*

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

## Results and discussions

### Effect levels

| | |
|---|---|
| **Endpoint** | no NOAEC identified |
| **Effect level** | |
| **Sex** | |
| **Basis for effect level / Remarks** | Test substance at a level of 30 mg/$m^3$ induced generally mild changes, wich quickly recovered during the exposure period. See Details on result. |

### 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
No particular observations
No mortality

BODY WEIGHT AND WEIGHT GAIN

Slightly decreased body weight, ~ -5 % by 13 wks exposure (Tab. 6)
Recovery: no significant difference from control at day 455, still ~ -4 % (52 weeks post-exposure)

HAEMATOLOGY
No significant effects, but white blood cell count elevated in both males and females at the end of exposure period , but not clearly attributable to increases in the numbers of neutrophilic leukocytes.After 13 weeks of recovery (day 176/177, Table 8/Table 13), neutrophil count still tended to be higher than the control in males and females, and normalized by 26 weeks of recovery (day 274/275, Table 9/Table 14).

CLINICAL CHEMISTRY
no significant effects

URINALYSIS
no significant effects

ORGAN WEIGHTS
No changes in heart, thyroid, adrenals, testes, brain, spleen, kidney Increased organ weights of lung and thymus at the end of exposure. Swollen lungs and enlarged mediastinal lypmph nodes

LUNG
Slight mean increase in relative weight: 1.3x (males, females) as compared to control [Tables 47 / 52]

LYMPH NODE: no weight data
PATHOLOGY
Swollen and spotted lungs and enlarged mediastinal lymph nodes.The effects gradually subsided after the exposure period:Lung weight normalised after 13 weeks post-exposure in males and females [Table 48 / 53].

HISTOPATHOLOGY: NON-NEOPLASTIC
In the lung (Table 68): Accumulation of alveolar macrophages, intra-alveolar polymorphonuclear leukocytes, and increased septal cellularityin malesand females. Treatment-related microscopic changes in the nasal region were occasionally found at the end of exposure period, such as very slight focal necrosis and slight atrophy of the olfactory epithelium, intracytoplasmic proteinaceous droplets. Accumulation of macrophages was seen in the mediastinal lymph nodes (disappeared after wk 39 post-exposure). Collagen content in the lungs was slightly increased at the end of exposure.During the recovery period all changes disappeared mostly within 13 to 26 week.

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 0.5 mg per lung of male animal groups, 0.35 mg per lung of female groups, decreasing over time and no longer measurable after 39 weeks post exposure (day 370).

## *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** Aug. 2000 - Feb. 2001

**Reliability** 1 (reliable without restriction)

**Rationale for reliability** GLP guideline study: Main study of a comparative study including three synthetic amorphous silicas

### Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Arts JHE, Muijser H, Kuper CF, Junker K | **Year** | 2003 |
| **Title** | A repeated 5-day inhalation study in rats, including two recovery periods, with synthetic amorphous silicas | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | TNO Chemistry, Zeist/NL | **Report no.** | V 2993 |
| **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, but willing to share

### Materials and methods

### Test type

subacute

### Limit test

no

### Test guideline

**Qualifier** according to

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

**Deviations** yes Only 5 exposure days; 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).

**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-200.

### Details on test material

CAS name, Silica, precipitated, crystalline-free; CAS No. 112926-00-8, Impurities: Na (1.9 %), S (0.8 %), Al (0.045 %), Fe (0.02 %), Ca 0.06 %

### Confidential details on test material

The test substance is equivalent to NM-200.

### Test animals

### Species

rat

### Strain

Wistar

### Sex

male/female

## Administration / exposure

### Route of administration

inhalation

### Type of inhalation exposure

nose only

### *Details on inhalation exposure*

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: 1.16 (± 0.36), 5.39 (± 0.58), 25.2 (± 1.5) (and for the control group receiving crystalline silica 24.4 (± 2.9) $mg/m^3$) [Appendix 1.1, Tab. 1.1]

### 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) = 2.83, 3.23, 3.27, and for the reference group 2.08 µm.[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 and females additionally, satellite groups of 10 each per sex 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.

**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
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 CONTENTSi content of the lung and tracheobronchial lymph nodes were determined.

HYDROXY OPROLINE 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: "Observation and examinations..."

***Statistics***

Various procedures acc. to the parameters under test (Report, p. 19/20)

## Results and discussions

### Effect levels

| | |
|---|---|
| **Endpoint** | NOEC mean (not any effects observed) |
| **Effect level** | 1.16 mg/m³ air (analytical) |
| **Sex** | male/female |
| **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") |
| **Endpoint** | NOAEC mean |
| **Effect level** | 5.39 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") |
| **Endpoint** | LOAEC mean |
| **Effect level** | 25.2 mg/m³ air (analytical) |
| **Sex** | male/female |
| **Basis for effect level / Remarks** | |

### Observations

***Clinical signs and mortality***

yes

***Body weight and weight gain***

no effects

***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 of TOXICITY:
None particular, except transient decreased breathing frequency. No mortality.

BODY WEIGHT:
normal

FOOD CONSUMPTION / FOOD EFFICIENCY:
No changes found (Tab. 4.1 / 4.2).

LUNG WEIGHT AND LYMPH NODES:
Slight increases in lung weights of the high-dose group, statistically significant absolute weights in males and relative weights in females, increase in relative weights of tracheobronchial lymph nodes in females of the high-dose group.

CELL DIFFERENTIATION IN LAVAGE:
No treatment-related changes were seen in the low-dose group (1.16 mg/$m^3$). Dose-related stimulation of neutrophil granulocytes: After 5 d, the absolute numbers of neutrophils increased significantly in the high-dose groups of both genders (Tab. 5.1) ($p<0.01$), the relative (not the absolute) number of macrophages decreased concomittantly (Tab. 6.1). In the mid-dose, too, neutrophils slightly increased (Tab. 5.1): This trend was confirmed by distinct positive shifts of the relative neutrophil counts (Tab. 6.1) ($p<0.01$ for males. (note: The statistical significance is not indicated for females in Tab. 5.1 + 6.1, although to be assumed).After recovery of 1 months (Tab. 5.2), the cell stimulating effects passed away again and were also absent after 3 months recovery in females, but noted in males without changes in absolute numbers (Tab. 5.3 + 6.3). Slight trends were also seen in the mid-dose group, but only reflected in the relative neutrophil increases, and just at the margin of statistical significance for the male group (5

mg/m$^3$) (Tab. 6.1). Note: In the reference group (crystalline silica), it was characteristic that in time-related, delayed fashion, the relative and absolute numbers of neutrophilsand macrophages significantly increased, more pronounced in males (Tab. 5.3 + 6.3).

BIOCHEMICAL PARAMETERS IN LAVAGE:
Significant increases in enzymes and protein levels were found only at the high-dose exposure, which completely reversed after recovery (Tab. 7).TNF-alpha showed no difference from the control in any group. The OH-proline content revealed no treatment-related changes.

MACROSCOPIC EXAMINATION:
no particular findings

HISTOPATHOLOGICAL EXAMINATION:
Histologically manifested changes were
- hypertrophy and hyperplasia of the brochiolar epithelium in 1/5 males and 2/5 females (high dose). No case occurred in the recovery groups. Because of the very rare occurrence in rats of that age, this lesion was considered treatment-related.
- very slight to slight polymorphonuclear leukocyte infiltration (inflammation response) at all dose levels, but not in the concurrent controls (Tab. 10.1). The incidence and severity was not clearly dose-related, 1/5 very slight case at the low dose level in the male and female group, respectively. This effect was occasionally observed in the recovery groups, but also in the recovery control groups to the same extent (Tab. 10.2). The authors considered this lesion to be unrelated to exposure. In recovery high-dose groups, tendency of accumulation of alveolar macrophages and hyperemic capillaries, unusual type-II hyperplasia in 1/5 males (Tab. 10.3).
- Note: In the reference group (crystalline silica), it was characteristic that in time-related, delayed fashion, an inflammatory reaction in all male and female rats emerged after 3-months recovery (alveolar cell infiltrates, alveolar cell debris, (multi)focal perivascular interstitial mononuclear cell infiltra tes, accumulation of macrophages). Macrophage aggregates found in mediastinal lymph nodes.

SILICON CONTENT:
One day after exposure, 30 - 40 µg Si were analysed in lungs of high-dose animals, which was below detection limit after 1 month recovery (<25 µg).On the contrary, in the crystalline silica group, Si accumulation was 4-5x higher (150 - 160 µg) and still persisted on a high level after recovery of 1 month (80 µg in females, 140 µg in males). [note: no determinations carried out in the low and mid-dose groups ]. No increased Si levels were observed in the lymph nodes in any group tested.

## Overall remarks, attachments

### Overall remarks

The high exposure concentration (25.2 mg/m$^3$) induced substance-related effects which reflect an inflammatory response of the lung tissue associated with morphological tissue reaction. These tend to disappear during recovery, but apparently not completely, but show clear signs of reversibility. Effects in the mid exposure concentration (5.39 mg/m$^3$) were confined to a very slight increase in the relative neutrophil count with concomitant decrease in the relative macrophage count at the day after exposure, but only statistically significant in males. There were no morphological tissue changes. No effects were noted at the low-concentration level (1.16 mg/m$^3$, analytical). It is concluded that the NOEC (acute/sub-acute) is at 1.16 mg/m$^3$. The NOAEC could be defined as 5.39 mg/m$^3$.

### 7.5.3 Repeated dose toxicity: dermal

### 7.5.4 Repeated dose toxicity: other routes

## 7.6 Genetic toxicity

### 7.6.1 Genetic toxicity in vitro

### *Endpoint study record: Genetic toxicity in vitro.001 FhG-ITEM 2912 Comet*

#### 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) |
| **Rationale for reliability** | GLP guideline study |

#### Data source

#### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Ziemann Ch, Knebel J | **Year** | 2012 |
| **Title** | Measurement of the DNA-damaging and cytotoxic potential of synthetic amorphous silica (NM-200) in cultured primary rat alveolar macrophages | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany | **Report no.** | 17G11540 |
| **Owner company** | CEFIC, Brussels/Belgium | | |
| **Company study no.** | | **Report date** | 2012-07-30 |

#### 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** no guideline available

**Guideline**

**Deviations**

## Principles of method if other than guideline

The Comet assay was conducted following the valid standard operating procedures of Fraunhofer ITEM and the principles outlined in: Tice et al. (2000) Single Cell Gell/Comet assay: Guidelines for In Vitro und In Vivo Genetic Toxicology Testing. Environ. Mol. Mutagen. 35(3):206-221. The used hOGG1-modified variant of the Comet assay is based on Smith et al. (2006): hOGG1 recognizes oxidative damage using the comet assay with greater specificity than FPG or ENDOIII. Mutagenesis 21 (3):185-190. In this case, the BAL (bronchoalveolar fraction) from rat lung was used as source for the test system of primary cell culture.

## 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

- Name of test material (as cited in study report): NM-200 (Synthetic amorphous silica)
- CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8- Substance type: inorganic
- Physical state: solid, white powder- Analytical purity: 96.5 % ($SiO_2$)- Ignition loss: 8.9 %
- Specific surface (BET): BET 230 $m^2$/g- Lot/batch No.: Master batch
- Stability under test conditions: stable
- Storage condition of test material: Ambient temperature, dry place, closed container, in the dark

## Method

## Species/strain

**Species/strain** primary culture, other: rat alveolar macrophages

**Details on** Lungs of untreated rats were lavaged (bronchoalveolar lavage, BAL) five times, each time

| | |
|---|---|
| **mammalian cell lines (if applicable)** | with 5 mL of 0.9 % NaCl (room temperature). Cells were recovered by centrifugation (256 x g for 10 min at 4 °C), re-suspended in growth medium (see Report 2.2) and counted. The resulting BAL cells were then plated at a density of 1.5 x 10^5 cells in 0.5 ml of growth medium per well on 24-well plates with hydrophobic culture surface. |
| **Additional strain characteristics** | other: Wistar rats (male, strain Crl:WU, age about 12 weeks) |
| **Metabolic activation** | not applicable |
| **Metabolic activation system** | without external rat liver microsomal fraction (S9) |

### Test concentrations

NM-200 - relevant test concentrations: 10, 50, and 250 ng/cm$^2$ (19, 95, and 475 ng/mL) [area dose derived from realistic in-vivo deposition in animal inhalation testing] NM-200
- additional concentrations, used for 4-h and 24-h exposure, respectively: 10 µg/cm$^2$ (19 µg/mL) and 2.5 µg/cm$^2$ (4.75 µg/mL) comparable to concentrations of controls
Control/reference substances: Aluminium oxide ($Al_2O_3$): 10 and 2.5 µg/cm$^2$ for 4-h and 24-h exposure, respectively. Quartz DQ12: 25 µg/cm$^2$ Potassium bromate ($KBrO_3$): 0.5 mM

### *Vehicle*

- Vehicle(s)/solvent(s) used: none, standard incubation medium (Dulbecco's-minimum essential medium (D-MEM) supplemented).

### Controls

| | |
|---|---|
| **Negative controls** | yes $Al_2O_3$ (Aluminium oxide) [CAS No. 1344-28-1] (particle size: geometrical mean, weighing by mass: 4.09 ± 1.77 µm) |
| **Solvent / vehicle controls** | no |
| **True negative controls** | yes Standard growth medium (background control) |
| **Positive controls** | yes middle size Dörentrup (87% alpha-quartz, 13% amorphous silica) [CAS No. 99439-28-8] (particle size: geometrical mean, weighing by mass: 2.99 ± 1.53 µm) |
| **Positive control substance** | other: Quartz DQ12 and potassium bromate ($KBrO_3$) |
| **Remarks** | |

### *Details on test system and conditions*

PRINCIPLE
In this study cultured primary rat alveolar macrophages from bronchoalveolar lavage (BAL) were used as a model system to investigate in parallel DNA-damage (hOGG1-modified Comet assay) and membrane integrity/cytotoxicity (LDH-release) of NM-200 in vitro. DNA-strand breaks and oxidative DNA damage

(8-OH-dG) were analysed in primary rat alveolar macrophages after 24 h of pre-culture and 4 or 24 h of incubation with the test and reference items, using the human 8 hydroxyguanine DNA-glycosylase 1 (hOGG1)-modified alkaline Comet assay, based on the alkaline version of the Comet assay. The single-cell gel (SCG)/Comet assay represents a test principle for identifying agents with genotoxic activity in mammalian cells and for further characterizing types of DNA damage. The assay is based on electrophoretic mobility of DNA fragments in agarose gels on slides. Evaluation unit is the single cell. The alkaline version (pH>13) of the Comet assay is able to detect DNA single- (SSB) and double-strand breaks (DSB), DNA-DNA and DNA-protein cross-links, alkali-labile sites (ALS) and SSBs associated with incomplete DNA excision repair. DNA damage is detected as DNA mi¬grating out of the cell nucleus during single-cell electrophoresis, resembling a comet tail. Tail length and tail intensity are proportional to the number of DNA strand breaks.

METHOD OF APPLICATION:
In medium on microtiter plates

DURATION
- Preincubation period: 20 - 24 h in growth medium
- Exposure duration: 24 h (test substance and $Al_2O_3$), 4 h (quartz and $KBrO_3$, time reduced because of high cytotoxcity)
- Fixation time (start of exposure up to fixation or harvest of cells): 24 h

CELL VIABILITY
Loss of lactate dehydrogenase (LDH): Changes of LDH activity were determined in the culture supernatants of the Comet assay (see Report 4.4) to be able to differentiate between unspecific DNA-damaging effects due to cytotoxic activity and particle specific DNA-damage. As medium control (background control), wells without cells, but containing growth medium were used. As the high control, BAL cells were treated for about 10 – 15 min with 1 % Triton X 100 to determine the maximum amount of releasable LDH-enzyme activity and to calculate % LDH-release/cytotoxicity. After the end of exposure, aliquots of 600 µl of the incubation media were taken for the LDH-assay and the macrophages were then placed on ice for 10 min to enable cell detachment without trypsination. LDH activity in the supernatant is measured by conversion of a tetrazolium salt to formazan (490 nm) which is formed proportionally to the amount of necrotic cells.

NUMBER OF REPLICATIONS: 2

DETERMINATION OF CYTOTOXICITY
- Method: other: see above LDH release

OTHER EXAMINATIONS
Oxidative DNA damage (Report 4.4: hOGG1-modified Comet assay) expressed by the formation of 8-hydroxy-2-deoxy-guanosine (8-OHdG), an important marker of DNA base oxidation. After treatment of with human 8 hydroxyguanine DNA-glycosylase 1 (hOGG1) followed by electrophoresis, the DNA was stained with ethidium bromide and analyzed for DNA fragmentation. As the main endpoint, the tail intensity of 100 nuclei per slide and treatment (with or without hOGG1 incubation) was determined per experiment with 3 independent experiments in total. The tail intensity is a direct measure for the number of broken pieces that can be standardized among various studies. A significant increase in tail intensity on the hOGG1-treated slides, as compared to the slides treated with enzyme buffer only, is indicative for the occurrence of the oxidative base lesion 8-OHdG.

### Any other information on materials and methods incl. tables

Particle size distribution (see Report, Appendix 4):x10.3= 3.9 µm; x50.3= 8.1 µm; x90.3= 16.5 µm *(*rounded to the first decimal place)
Dispersion technique: Stirring for 24 h with magnetic stirrer (no further details)
Analysis: by Dynamic Light Scattering (DLS) / laser light diffraction analysis using a Zetasizer-NanoZS instrument of Malvern.Solubility in medium (see Report, Appendix 4):83 - 90 mg $SiO_2$/L (Loading 5 g/L)
Dispersion technique: Stirring for 24 h with magnetic stirrer (no further details).
Analysis: ICP OES (inductively coupled plasma optical emission spectroscopy)

## Results and discussions

### Test results

| | |
|---|---|
| **Species/strain** | primary culture, other: rat alveolar macrophages |
| **Metabolic activation** | not applicable |
| **Test system** | all strains/cell types tested |
| **Genotoxicity** | negative |
| **Cytotoxicity** | no absent at the test concentrations (considered relevant in vivo), but found at 10 µg/$cm^2$ (= 19 µg/mL). |
| **Vehicle controls valid** | not applicable |
| **Negative controls valid** | yes |
| **Positive controls valid** | yes |

***Additional information on results***

TEST-SPECIFIC CONFOUNDING FACTORS
- Effects of pH: no - Effects of osmolality: no

### Remarks on results including tables and figures

see attachment

## Overall remarks, attachments

### Overall remarks

In the present study, the synthetic amorphous silica, NM-200, under the test conditions, and in the cell type used (BAL cells, pre-cultured for 24 h) did not induce either DNA-strand breaks or oxidative DNA-base lesions at the chosen concentrations relevant for the rat lung in vivo, both after 4 and 24 h of incubation. Only at the highest concentration used (10 µg/$cm^2$, 4 h of incubation) a very slight, but statistically significant increase in tail intensity was noted, when compared to the negative control. However, if compared to the particulate negative control (same mass concentration of 10 µg/$cm^2$), no significant increase in the tail intensity (TI value) was obvious. Absence of significance for the particulate negative control indicates that the biological relevance of the observed small effect is questionable and represents more likely a particle and not a material-specific effect or is of incidental nature. This is

supported by the facts that the negative control in the present study exhibited a very low mean TI with a very small SD value and that the mean TI of 0.9 % at 10 µg/cm$^2$ lay within the range of historical negative controls.

### Attached background material

**Attached document** FhG 2912 Genotoxic effects.pdf / 84.59 KB (application/pdf): ENV/JM/MONO(2015)14/ANN10

**Remarks**

### Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

Due to the absence of significant increases in the tail intensity in NM-200 treated BAL cells, as compared to the particulate negative control ($Al_2O_3$), the amorphous silica, NM-200, did not show a significant clastogenic potential or a potential to induce oxidative DNA base lesions (8-OH-dG) in primary rat alveolar macrophages, both after 4 and 24 h of incubation.

## *Endpoint study record: Genetic toxicity in vitro.002 FhG 2012 MLA*

### 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)

**Rationale for reliability** GLP guideline study

### Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Ziemann Ch, Knebel J | **Year** | 2012 |
| **Title** | In Vitro Mammalian Cell Gene Mutation Test in Mouse Lymphoma L5178Y/TK+/- Cells with Synthetic Amorphous Silica (NM-200) | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany | **Report no.** | 17G11007 |
| **Owner company** | CEFIC, Brussels/Belgium | | |
| **Company study no.** | | **Report date** | 2012-06-14 |

### 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**

**Qualifier** according to

**Guideline** EU Method B.17 (Mutagenicity - In Vitro Mammalian Cell Gene Mutation Test)

**Deviations**

### Principles of method if other than guideline

The test was conducted by using microtiter plates: In this case , a so-called "Global Evaluation Factor", based on historical control data of a broad range of laboratories, was set at 125 mutants above background: i.e. a relevant increase in the mutation frequency (MF) is stated, if the MF of the test substance amounts to more than (MF of negative/vehicle control + 125) (Moore et al. 2003: Mutation Research 540, 127-140).

### 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

- Name of test material (as cited in study report): NM-200 (Synthetic amorphous silica)
- CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8
- Substance type: inorganic
- Physical state: solid, white powder
- Analytical purity: 96.5 % ($SiO_2$)
- Ignition loss: 8.9 %
- Specific surface (BET): BET 230 $m^2$/g-
  Lot/batch No.: Master batch
- Stability under test conditions: stable
- Storage condition of test material: Ambient temperature, in the dark under $N_2$

### Method

### Target gene

thymidine kinase locus

### Species/strain

| | |
|---|---|
| **Species/strain** | mouse lymphoma L5178Y cells |
| **Details on mammalian cell lines (if applicable)** | - Type and identity of media: HEPES-buffered RPMI-1640 medium - Periodically checked for Mycoplasma contamination: yes- Periodically checked for karyotype stability: yes- Periodically "cleansed" against high spontaneous background: yes |
| **Additional strain characteristics** | other: clone 3.7.2C |
| **Metabolic activation** | with and without |
| **Metabolic activation system** | Induced rat liver S9: pretreatment with phenobarbital (80mg/kg bw, 3x i.p.) and ß-naphtoflavone ((80mg/kg bw, 3x orally), each on consecutive days). |

### Test concentrations

Two independent tests with and without S9 mix: 10; 100; 300; 900; 2700, and 5000 µg/mL

### *Vehicle*

none, standard culture medium

### Controls

| | |
|---|---|
| **Negative controls** | yes Standard culture medium |
| **Solvent / vehicle controls** | no |
| **True negative controls** | no |
| **Positive controls** | yes in the absence of S9 |
| **Positive control substance** | methylmethanesulfonate |

| | |
|---|---|
| **Remarks** | |
| **Negative controls** | no |
| **Solvent / vehicle controls** | yes |
| **True negative controls** | no |
| **Positive controls** | yes in the presence of S9 |
| **Positive control substance** | cyclophosphamide |
| **Remarks** | |

### *Details on test system and conditions*

METHOD OF APPLICATION: in medium
PREPARATION of STOCK SOLUTION
10 g/L of the sterilised test substance (two fold the limit concentration of 5 g/L) was suspended in standard culture medium (containing 5 % instead of 10 % heat-inactivated horse serum), also called incubation and washing medium. The suspension was stirred for 24 h at room temperature to minimise agglomerisation. Despite aggregation homogeneous dosing was possible.

DURATION
- Exposure duration: 4 hours with and without metabolic activation
- Expression time (cells in growth medium): 48 hours (6 *10^6 cells)
- Selection time (if incubation with a selection agent): 14 days

SELECTION AGENT (mutation assays):

RPMI 1640 medium by addition of 3 µg/mL TFT (5-Trifluorothymidine)

NUMBER OF REPLICATIONS: 2

NUMBER OF CELLS EVALUATED:
For selection - 2*10^3 cells per well on four 96-well plate per treatment group. TFT-resistant colonies were evaluated microscopically at 40-fold magnification 14 days after plating. The number of positive and negative wells was recorded, together with the total number of countable wells (384 per treatment group and experiment, 768 in total). Colonies seen in the TFT-selection plates were qualified as large and small colonies, and the number of large and small colonies was also recorded.

DETERMINATION OF CYTOTOXICITY
- Method: Suspension growth (SG), relative total growth (RTG) and PE (plating efficiency) ± S9

### *Evaluation criteria*

A study is judged as valid, if the following criteria are fulfilled:
• Cells exhibited normal morphology.
• SG of the negative/vehicle controls amounted to values between about 8 and 32.
• PE viable of the negative/vehicle controls (calculated from survivor II data) was not < 0.65 or > 1.2.
• There was a minimum of 4 analyzable concentrations with mutant frequency data.
• Spontaneous mutant frequencies were within the historical range or the range (50 - 200 x 10^-6) as proposed by Moore et al. (2003).
• At least one concentration of each positive control (with and without S9-mix) exhibited significant increase of mutant frequency with regard to the background level.

• The colony size distribution for the MMS positive control showed an increase in both small and large colonies (Moore et al., 1985; Aaron et al., 1994).Evaluation of mutagenicity of test results:
• The result is positive, if the induced mutation frequency reproducibly exceeds a threshold of 125 colonies per 10^6 cells above the corresponding solvent or negative control, resp. (125 = "Global Evaluation Factor": Moore et al. 2003). A relevant increase in the mutation frequency (MF) is stated, if MF of the test substance amounted to more than (MF of negative/vehicle control + 125).
• A relevant increase of the mutation frequency should be dose-dependent.
• Whenever a test substance is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. References: - Aaron et al. 1994: Mammalian cell gene mutation assay working group report. Mut. Res. 312, 235-239- Moore et al 1985: In situ analysis of trifluorothymidine-resistant (TFT) mutants of L5178Y/TK+/- mouse lymphoma cells. Mut. Res. 151, 147-159- Moore et al. 2003: Mouse lymphoma thymidine kinase gene mutation assay: Inter. Workshop on Genotoxicity Test, Workgroup Report-Plymouth, UK 2002.Mut. Res. 540, 127-140

### *Statistics*

not relevant: Only if there is a relevant increase in one or more than one dose group, dose-dependency is further tested by linear trend analysis.

### Any other information on materials and methods incl. tables

Particle size distribution (see Report 8.1 and Appendix 4):
Hydrodynamic diameters: x10.3 = 4.2 µm; x50.3 = 9.6 µm; x90.3 = 20.9 µm * (* rounded to the first decimal place)

Dispersion technique: Stirring for 24 h with magnetic stirrer (no further details) Analysis: by Dynamic Light Scattering (DLS) / laser light diffraction analysis using a Zetasizer-NanoZS instrument of Malvern.
Solubility in medium (see Report 8.1 and Appendix 4):103 - 105 mg $SiO_2$/L (Loading 5 g/L)
Dispersion technique: Stirring for 24 h with magnetic stirrer (no further details)

Analysis: ICP OES (inductively coupled plasma optical emission spectroscopy)

## Results and discussions

### Test results

| | |
|---|---|
| **Species/strain** | mouse lymphoma L5178Y cells |
| **Metabolic activation** | with and without |
| **Test system** | all strains/cell types tested |
| **Genotoxicity** | negative |
| **Cytotoxicity** | yes incipient at 2.7 mg/mL; but marked at highest concentration (5 mg/mL) |
| **Vehicle controls valid** | not applicable |
| **Negative controls valid** | yes |
| **Positive controls valid** | yes |

### *Additional information on results*

TEST-SPECIFIC CONFOUNDING FACTORS
- Effects of pH: no, only increase of 0.3 - 0.4 units on addition of 5000 µg/ml and 4 h incubation

- Effects of osmolality: no
- Evaporation from medium: no
- Water solubility: no, poorly soluble
- Precipitation: The presence of particles may have influenced cell viability at high dosage
- Other confounding effects: possibly direct interaction of particles with the cells (see cell toxicity at highest concentration: Adsorption of the test item aggregates/agglomerates to the cells may also explain the apparently lower cytotoxic potential of the limit concentration by analysis of the survivor II plates.)

### Remarks on results including tables and figures

see attachment

### Overall remarks, attachments

### Attached background material

**Attached document** FhG 2012 Genotoxic effects.pdf / 31.72 KB (application/pdf): ENV/JM/MONO(2015)14/ANN10

**Remarks**

### Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

Synthetic amorphous silica (NM-200), both with and without S9-mix, induced no relevant increase in the mutation frequency (MF). Therefore, the test substance is not mutagenic in the mouse lymphoma L5178Y/TK+/- cells under the restriction of the assay and the test conditions used.

## *Endpoint study record: Genetic toxicity in vitro.003 FhG-ITEM 2012 CA*

### 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) |
| **Rationale for reliability** | GLP guideline study |

## Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Ziemann Ch, Knebel J | **Year** | 2012 |
| **Title** | In Vitro Mammalian chromosome Aberration Test (V79 Cells) with Synthetic Amorphous Silica (NM-200) | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany | **Report no.** | 17G11019 |
| **Owner company** | CEFIC, Brussels/Belgium | | |
| **Company study no.** | | **Report date** | 2012-07-20 |

### 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**

### 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

- Name of test material (as cited in study report): NM-200 (Synthetic amorphous silica)
- CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8
- Substance type: inorganic
- Physical state: solid, white powder
- Analytical purity: 96.5 % ($SiO_2$)
- Ignition loss: 8.9 %
- Specific surface (BET): BET 230 $m^2/g$
- Lot/batch No.: Master batch
- Stability under test conditions: stable
- Storage condition of test material: Ambient temperature, in the dark under $N_2$

### Method

### Species/strain

| | |
|---|---|
| **Species/strain** | Chinese hamster lung fibroblasts (V79) |
| **Details on mammalian cell lines (if applicable)** | - Type and identity of media:- Properly maintained: yes (gas phase of liquid nitrogen)<br>- Periodically checked for Mycoplasma contamination: yes |
| **Additional strain characteristics** | no data |
| **Metabolic activation** | with and without |
| **Metabolic activation system** | Induced rat liver S9: pretreatment with phenobarbital (80mg/kg bw, 3x i.p.) and ß-naphtoflavone ((80mg/kg bw, 3x orally), each on consecutive days). |

### Test concentrations

Concentrations effectively evaluatged (see Report 14, Tables 4, 5, and 6): 4 h, without S9: 0, 100, 200, 600, 1800 µg/mL 4 h, with S9: 0, 600, 1000, 1500 µg/mL24 h, without S9: 0, 2, 5, 16, 48 µg/mL

### *Vehicle*

- Vehicle(s)/solvent(s) used: none, standard incubation medium (Dulbecco's-minimum essential medium (D-MEM) supplemented)

### Controls

| | |
|---|---|
| **Negative controls** | yes standard incubation medium |
| **Solvent / vehicle controls** | no |

| | |
|---|---|
| **True negative controls** | no |
| **Positive controls** | yes without S9 |
| **Positive control substance** | ethylmethanesulphonate |
| **Remarks** | |
| **Negative controls** | yes standard incubation medium |
| **Solvent / vehicle controls** | no |
| **True negative controls** | no |
| **Positive controls** | yes with S9 |
| **Positive control substance** | cyclophosphamide |
| **Remarks** | |

***Details on test system and conditions***

METHOD OF APPLICATION: in medium
NM-200 was sterilized by heating to 180°C for 1 h. The test substance was then accurately weighed, and the stock suspensions were prepared in the respective incubation media (see Report 8.2.1 and 8.3.1) with 10 % or 2% FBS, respectively. To minimize agglomeration of the NM-200 particles, the stock solutions were stirred for 24 h at room temperature prior to cell incubation. This procedure could not avoid agglomeration of existing test substance aggregates, but enabled homogeneous dosing of thesuspensions. Dilutions of the stock suspensions were subsequently made by using the respective incubation media, resulting in test substance suspensions at desired concentrations. Prior to cell exposure, the diluted test substance suspensions and the reference item-containing incubation media were vortexed shortly to ensure homogeneity.

DOSE SELECTION (Report 8.10):
At least 3 evaluable concentrations (separated by no more than a factor between 2 and 10^0.5) of the test substance are needed per treatment (short term without and with S9-mix and long). If cytotoxicity occurs in the pre-experiment, the 2 highest concentrations of the test item used in the main experiments should be in the toxic range of the dose-response curve. The highest concentration should show a significant reduction (~50% or more) in the M.I., as compared to the respective vehicle control. However, the highest concentration should still ensure occurrence of enough metaphases for analysis of chromosomal aberrations.

DURATION
- Preincubation period: 24 h
- Exposure duration: 4 and 24 h without S9-mix and 4 h with S9-mix
- Expression time (cells in growth medium): For 4h exposures, time to approx. 1.5 of a normal cell cycle (approx. 21 h)
- Fixation time (start of exposure up to fixation or harvest of cells): 24 h

SPINDLE INHIBITOR (cytogenetic assays): Colcemid

STAIN (for cytogenetic assays): Giemsa

NUMBER OF REPLICATIONS: 2

NUMBER OF CELLS EVALUATED:

100 well spread metaphases per culture (A and B), 200 metaphases per treatment

DETERMINATION OF CYTOTOXICITY
- Method: mitotic index

EXAMINATIONS on ABERRATIONS
- Gaps (g = chromatid and G = isochromatid)
- Breaks (b = chromatid and B = isochromatid)
- Chromatid type exchanges (ex)
- Chromosome type exchanges (EX)
- Endoreduplications (e)
- Aneuploidy (for example polyploidy = py
- Complete metaphase pulverization (ma)

### *Evaluation criteria*

Evaluation of mutation response: A test substance is considered to induce structural chromosome aberrations in cultured mammalian somatic cells (positive result), if the number of aberrant cells falls within the range of concurrent positive controls or if there is a dose-related increase in the percentages of aberrant cells in comparison to the concurrent negative/vehicle controls. The test substance is also considered to be positive, if a reproducible increase in the number of cells with aberrations occurs for at least one test condition. An increase in polyploid cells may indicate that the test item has the potential to inhibit mitotic processes and to induce numerical chromosome aberrations.The test substance is considered negative and not to induce chromosomal aberrations in cultured mammalian somatic cells (negative result), if there is no increase at any dose in the percentage of aberrant cells in comparison to concurrent negative/vehicle controls.

### *Statistics*

Statistical methods like the chi2 -test or Fisher's exact test may be used to evaluate positve or negative test results, but up to now no unequivocal statistical method has been developed for evaluating chromosomal aberrations.

### Any other information on materials and methods incl. tables

Particle size distribution (see Report 8.1 and Appendix 4):Experiments without S9-mix, 10% fetal bovine serum (FBS): Hydrodynamic diameters: x10.3= 3.9; µm, x50.3= 8.1 µm; x90.3= 16.5 µm *Experiments with S9-mix, 2% FBS: Hydrodynamic diameters: x10.3 = 4.7 µm; x50.3= 11.8 µm; x90.3= 33.5 µm * (*rounded to the first decimal place)Dispersion technique: Stirring for 24 h with magnetic stirrer (no further details) Analysis: by Dynamic Light Scattering (DLS) / laser light diffraction analysis using a Zetasizer-NanoZS instrument of Malvern.Solubility in medium (see Report 8.1 and Appendix 4):83 - 90 mg $SiO_2$/L (Loading 5 g/L) Dispersion technique: Stirring for 24 h with magnetic stirrer (no further details) Analysis: ICP OES (inductively coupled plasma optical emission spectroscopy)

## Results and discussions

### Test results

| | |
|---|---|
| **Species/strain** | Chinese hamster lung fibroblasts (V79) |
| **Metabolic activation** | with and without |
| **Test system** | all strains/cell types tested |
| **Genotoxicity** | negative |
| **Cytotoxicity** | yes marked at ≥ 500 mg/mL (4 h, -S9); at > 1000 µg/mL (4 h, +S9); at ≥ 10 µg/mL (24 h, -S9) (see Report 14, Tables 1A, 1B, and 2, 3). |
| **Vehicle controls valid** | not applicable |
| **Negative controls valid** | yes |
| **Positive controls valid** | yes |

***Additional information on results***

TEST-SPECIFIC CONFOUNDING FACTORS
- Effects of pH: no, slight increase in pH from 7.40 to 7.63 (at 5000 mg/mL)
- Effects of osmolality: no
- Evaporation from medium: no
- Water solubility: no, only slightly soluble
- Precipitation: Suspension tested (see cytotoxicity)

ADDITIONAL INFORMATION ON CYTOTOXICITY:
NM-200 demonstrated clear concentration-dependent cytotoxicity in the short term experiments, as determined by the mitotic index (M.I.). Cytotoxicity was also observed after 24 h of incubation and was more pronounced than after 4 h of incubation.

**Remarks on results including tables and figures**

see attachment

## Overall remarks, attachments

### Overall remarks

I

### Attached background material

**Attached document** FhG 2012 Genotoxic effects CA.pdf / 36.42 KB (application/pdf): ENV/JM/MONO(2015)14/ANN10

**Remarks**

## Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

Due to the low aberration frequencies in NM-200 treated cultures, which in the case of short term exposures fell in the range of historical negative controls, synthetic amorphous silica (NM-200) did not induce structural chromosomal aberrations in cultured mammalian somatic cells under the test conditions used.

## *Endpoint study record: Genetic toxicity in vitro_NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

## 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

5/20/40/ 80/100 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 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_NM-200_COMET 16 HBE_UAB.docx / 22.88 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

## Applicant's summary and conclusion

### Interpretation of results

other: -Without FpG Positive at 3 h: Dose-dependant increase in the % Tail DNA at 4 doses (20, 40, 80 and 100 µg/ml); Negative at 24 h. -With FpG: Negative at 3 and 24 h.

### Conclusions

SAS NM-200 induces DNA strand breaks in 16-HBE cells at 3 h but not 24 h treatment with the alkaline comet assay. SAS NM-200 does not induce oxidative DNA damage at both 3 h and 24 h at the tested dose with the FpG-modified alkaline comet assay

### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: Genetic toxicity in vitro_NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

## 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

## Applicant's summary and conclusion

### Interpretation of results

other: Without FpG: Equivocal at 3h: increase in the % Tail DNA at the lowest dose only (2.56 µg/ml); Negative at 24h. With FpG: Negative at both 3 and 24 h.

### Conclusions

SAS NM-200 induces equivocal genotoxic response in A 549 cells following 3h treatment and is not genotoxic following 24 h treatment at the tested dose with the alkaline comet assay.SAS NM-200 does not induce oxidative DNA damage at both 3 and 24 h at the tested dose with the FpG-modified comet

assay.

### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: Genetic toxicity in vitro_NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

## 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_NM-200_COMET BEAS-2B_IPH.docx / 23.82 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

other:
-Without FpG Positive at 3 h: increase in the % Tail DNA at 2 doses (25.6 and 256 µg/ml)
-With FpG (3 h only) Equivocal: increase in the % Tail DNA at 1 dose

### Conclusions

SAS NM-200 induces DNA strand breaks in BEAS-2B cells at 2 doses following 3 h treatment with the alkaline comet assay. SAS NM-200 induces equivocal oxidative DNA damage following 3 h treatment with the FpG-modified alkaline comet assay

### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: Genetic toxicity in vitro_NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### 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 hUse 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_NM-200_MN Caco-2_Anses.docx / 21.78 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

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

### Conclusions

SAS NM-200 induces DNA strand breaks at 2 doses in A 549 cells following both 3 h and 24 h treatment with the alkaline comet assay.SAS NM-200 induces oxidative DNA damage at 24 h and an equivocal response at 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-200_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-200

## 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_NM-200_MLA TK_IPL.docx / 19.64 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

SAS NM-200 is not mutagenic in L5178Y TK +/-mouse lymphoma cells at the tested dosesWith 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-200_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-200

### 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

8/16/32µg/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_NM-200_MN 16HBE_IPL.docx / 19.67 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

SAS NM-200 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-200_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-200

### 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

### Overall remarks, attachments

### Attached full study report

NGTX_gentox_invitro_NM-200_MN A549_INRS.docx / 20.62 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

negative No increase in the frequency of binucleated cells with micronuclei in both experiments

### Conclusions

SAS NM-200 does not induce aneugenic/clastogenic damage in A549 cells at the tested dose with the cytokinesis-block micronucleus assay

### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: Genetic toxicity in vitro_NM-200_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-200

### 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_NM-200_MN BEAS-2B_FIOH.docx / 19.66 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

SAS NM-200 does not induce aneugenic/clastogenic damage in BEAS-2B at the tested 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-200_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-200

## 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_NM-200_MN Caco-2_Anses.docx / 20.66 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

other: Statistical dose-dependant increase in the frequency of binucleated cells with micronuclei in 2 out 3 experiments

**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-200 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-200_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-200

### 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

64/128 and 256 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_NM-200_MNLympho_IPL.docx / 20.89 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

### Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

SAS NM-200 does not induce aneugenic/clastogenic damage in primary human blood lymphocytes cells at the tested dose cytokinesis-block micronucleus assay.

### Cross-reference to other study

http://www.nanogenotox.eu/

## 7.6.2 Genetic toxicity in vivo

## *Endpoint study record: Genetic toxicity in vivo_NM-200_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-200_MN Bone marrow Gavage and NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### Test animals

### Species

rat

### Strain

Sprague-Dawley

### Sex

male

### *Details on test animals and environmental conditions*

Each MN was tested separately. Experiments were conducted in male Sprague--dawley rats 6--8 weeks old (around 200 g). For each MN, the animals were divided into 5 groups with 5 animals per groups. Normal saline was used as a vehicle control whereas methylmethansulfonate (MMS) was used as a

positive control. The animals were treated via oral route (gavage) as three administrations at 0, 24 h and 45 h. In the case of MMS, the last dose was reduced to 80 mg/kg. Animals were sacrificed 3 h after the last administration.

## 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 hSampling 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_NM-200_COMET gavage_Anses.docx / 20.77 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-200 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-200 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-200_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-200_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-200

### Test animals

### Species

rat

### Strain

Sprague-Dawley

### Sex

male

### *Details on test animals and environmental conditions*

Seven-week old Sprague Dawley rats were purchased from Janvier and were exposed by intratracheal instillation to particle suspensions or vehicle 48, 24 and 3 hours before tissue collection. Three concentrations were tested 12; 6 and 3 mg/kg b.w.

## 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 hSampling: 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_NM-200_COMET instillation_Inrs.docx / 22.22 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 intratracheal instillation with SAS NM-200 irrespective of the organ investigated. Using FpG, no oxidative DNA damage was induced in the MN treated animals compared to the control.

### Conclusions

SAS NM-200 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-200_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-200_COMET gavage and NM-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### Test animals

### Species

rat

### Strain

Sprague-Dawley

### Sex

male

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

Each MN was tested separately. Experiments were conducted in male Sprague--dawley rats 6--8 weeks old (around 200 g). For each MN, the animals were divided into 5 groups with 5 animals per groups. Normal saline was used as a vehicle control whereas methylmethansulfonate (MMS) was used as a positive control. The animals were treated via oral route (gavage) as three administrations at 0, 24 h and 45 h. In the case of MMS, the last dose was reduced to 80 mg/kg. Animals were sacrificed 3 h after the last administration.

## 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 MethaneSulfonate (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_NM-200_MN Bone marrow_Gavage_Anses.docx / 20.12 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-200 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-200 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-200_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-200_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

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-200

### Test animals

### Species

rat

### Strain

Sprague-Dawley

### Sex

male

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

Seven--week old Sprague Dawley rats were purchased from Janvier and were exposed by intratracheal instillation to particle suspensions or vehicle 48, 24 and 3 hours before tissue collection. Three concentrations were tested 12; 6 and 3 mg/kg b.w.

### 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 hSampling: 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 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

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_NM-200_MN Bone marrow_instillation_Inrs.docx / 42.72 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-200 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-200 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-200_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-200_COMET Gavage and NM-200_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-200

## Test animals

## Species

rat

## Strain

Sprague-Dawley

## Sex

male

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

Each MN was tested separately. Experiments were conducted in male Sprague--dawley rats 6--8 weeks old (around 200 g). For each MN, the animals were divided into 5 groups with 5 animals per groups. Normal saline was used as a vehicle control whereas methylmethansulfonate (MMS) was used as a positive control. The animals were treated via oral route (gavage) as three administrations at 0, 24 h and 45 h. In the case of MMS, the last dose was reduced to 80 mg/kg. Animals were sacrificed 3 h after the last administration.

## 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 hSampling 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 MethaneSulfonate (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_NM-200_MN colon_Gavage_Anses.docx / 18.02 KB (application/octet-stream): ENV/JM/MONO(2015)14/ANN10

## Applicant's summary and conclusion

### Interpretation of results

negative

### Conclusions

SAS NM-200 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/

# 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** | key study (X) robust study summary ( ) used for classification ( ) used for MSDS |
| **Study result type** | experimental result **Study period** 05 Jan. - 19 Sep. 2011 |
| **Reliability** | 1 (reliable without restriction) |
| **Rationale for reliability** | GLP guideline study |

#### Data source

#### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Wolterbeek APM | **Year** | 2012 |
| **Title** | Oral two-generation reproduction study with NM-200 synthetic amorphous silica in Wistar rats (Vol. 1) | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | TNO Triskelion, Zeist/The Netherlands | **Report no.** | V9127 |
| **Owner company** | CEFIC (The European Chemical Industry Council ), Brussels/Belgium | | |
| **Company study no.** | LRI N3-TNO | **Report date** | 2012-12-11 |

#### Data access

other: data owner or letter of access

#### Data protection claimed

yes, but willing to share

#### Materials and methods

#### Test type

two-generation study

#### Test guideline

**Qualifier** according to

**Guideline** OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)

**Deviations**

### 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

- Test material form: dispersion- Name of test material (as cited in study report): NM-200 Synthetic Amorphous Silica
- CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8
- Substance type: inorganic- Physical state: solid, white powder
- Analytical purity: 96.5 % ($SiO_2$)
- Ignition loss: 8.9 %- particle size: Primary crystal size by TEM 20 nm (Report, under Annexes Certificate of analysis)
- Specific surface (BET): BET 230 $m^2$/g-
Lot/batch No.: PR-A-2
- Expiration date of the lot/batch: 25 Oct. 2011
- Stability under test conditions: stable
- Storage condition of test material: Ambient temperature, in the dark under $N_2$

## Test animals

### Species

rat

### Strain

Wistar

### Sex

male/female

### *Details on test animals and environmental conditions*

TEST ANIMALS

- Source: Charles River Deutschland, Sulzfeld, Germany
- Age at study initiation: (F0) 5 wks; (F1) 3 wks (after weaning)
- Weight at study initiation (mean of groups): (F0: Report Table 7) Males: 188 - 193 g; Females: 123 - 125 g; (F1: Report Table 8) Males: 664 - 660 - 62 g; Females: 60 - 62 g
- Fasting period before study: no
- Housing: during pre-mating 4 animals/sex/cage; during mating, 1 female + 1 male; post-mating, females individually; after weaning, 4 pups (F1)/sex/cage.
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 12 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ± 2 °C
- Humidity (%): 45 - 65 %
- Photoperiod (hrs dark / hrs light): 12 / 12

## Administration / exposure

### Route of administration

oral: gavage

### Vehicle

other: Methylhydroxypropylcellulose (Methocel F4M Food Grade Modified Cellulose)

#### *Details on exposure*

PREPARATION OF DOSING SOLUTIONS:VEHICLE
- Justification for use and choice of vehicle: non-toxic and well tolerable, stablising homogeneity of the silica dispersion
- Concentration in vehicle: 0.5 % (w/v) in water
- Amount of vehicle (if gavage): 10 mL/kg bw/d
- Frequency of preparation: Weekly by mixing 5 g Methocel in 1 L Ultrapure water on a magnetic stirrer for approx. 24 h, occasionally warmed up for a couple of hours to facilitate dissolution.
- Storage: 2 - 10°C

TEST SUBSTANCE SOLUTION
- Frequency of preparation: Weekly
- The required amount of vehicle was added to bottles containing the corresponding amount of the test substance under stirring on a magnetic stirrer (ca. 900 rpm), continued for at least 60 minutes. All samples were continuously stirred on a magnetic stirrer (ca. 900 rpm) during the entire daily administration period in order to maintain the homogeneity of the test substance in the vehicle (Report 4.5.2). The nominal concentrations were 10, 30, and 100 g/L.
- Particle size distribution in dispersion, measured separately by dynamic light scattering (Report 5.1 and Annex 6): The mean hydrodynamic diameters of the $SiO_2$ particles were found to vary between 1076-1664 nm (10 g/L), between 876-1216 nm (30 g/L), and between 409-703 nm (100 g/L). Due to the high concentration of the particles in the samples they sedimented and aggregated. Furthermore, multiple scattering occurred in these samples which also influenced the measurements.

### Details on mating procedure

- M/F ratio per cage: 1/1

- Length of cohabitation: ≤ 14 d
- Proof of pregnancy: sperm in vaginal smear, referred to as day 0 of pregnancy
.- Unsuccessful pairing replacement of first male by another male with proven fertility: no data, only if first male died during mating period.
- Further matings after two unsuccessful attempts: no (Females that did not show evidence of copulation after the end of the 2-weeks mating period were housed individually until sacrifice (more than 21 days after the last day of the mating period).
- After successful mating each pregnant female was caged individually: yes

## Analytical verification of doses or concentrations

yes

### *Details on analytical verification of doses or concentrations*

The test substance in the vehicle was analyzed by Dynamic Light Scattering (DLS) analysis using a Zetasizer-NanoZS instrument of Malvern (see Report Annex 6):This technique yields a hydrodynamic diameter that is calculated via the Stokes-Einstein equation from the DLS measurements, moreover resulting in peak values of the hydrodynamic diameter distribution as well as the polydispersity index (PDI) that describes the width of the particle size distribution.As dispersant methylhydroxypropylcellulose (Methocel F4M Food Grade Modified Cellulose) was used, based on a protocol of Fraunhofer institute ITEM (see 28-d study). In pre-tests, several dispersion techniques were employed: Magnetic stirrer and various US-sonification techniques. Vigorous stirring (1 h) followed by a lower stirring speed was ultimately used as appropriate dispersion method (Definitely, for the highly concentrated dispersion (100 g/L), the stirring time needed to be prolonged to 1 h.) The dispersion thus prepared could be shock-frozen in liquid nitrogen and be stored until use for testing.In the main animal study, particle size distribution, particle size stability and homogeneous distribution of the particles in the vehicle were recorded: At 5 different weeks during the study (during premating of F0-generation, start of mating F0-generation, start of F1-generation, start of mating F1-generation and last week of the study), samples were taken from each of the dosing formulations (including control) for analytical investigations.

## Duration of treatment / exposure

F0-Generation: The female animals were dosed during a 10-week premating period and during mating, gestation and lactation up to postnatal day 21.F1-Generation: Selected F1-generation pups were dosed from postnatal day 22 until the day prior to sacrifice.

## Frequency of treatment

1x/d

## Details on study schedule

- F1 parental animals not mated until 10 weeks after selected from the F1 litters (after premating phase).
- Selection of parents from F1 generation when pups were 21 - 27 days of age (after weaning).
- Age at mating of the mated animals in the study: 13 - 14 weeks (premating phase of 10 weeks plus lactation and selection phase after weaning)(see also Table 8)

## Doses / concentrations

0, 100, 300, and 1000 mg/kg bw/d

**Basis** other: nominal daily dose (in 10 mL/kg bw/d)

### No. of animals per sex per dose

initially 28 (F0- and F1-generation)

### Control animals

yes, concurrent vehicle

### *Further details on study design*

- Dose selection rationale: based on previous prenatal developmental toxicity study and 28-d oral toxicity study (Report, 4.4).

### *Positive control*

none

### Examinations

### *Parental animals: Observations and examinations*

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: 2x/day on working days, else 1x/day
- Cage side observations for the F0- and F1-generations are included in the Report, Tables 1-6, Appendices 3-8.

DETAILED CLINICAL OBSERVATIONS:
Yes, included above

BODY WEIGHT:
Yes, in Report, Tables 7-18, Appendices 9-14:
- Time schedule: Before the start of the treatment at randomization and at the start of the study (premating).Males were weighed weekly until sacrifice. Females were weighed weekly during the premating and mating period. Mated females were weighed on days 0, 4, 7, 10, 14, 17 and 21 during presumed gestation and on days 1, 4, 7, 10, 14, 17 and 21 of lactation.

FOOD CONSUMPTION AND COMPOUND INTAKE:
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes (Report, Tables 19-24), Appendices 15-20)

### *Estrous cyclicity (Parental animals)*

Vaginal smears to evaluate the estrus cycle length and normality were made daily for about 3 weeks prior to mating. Smears were made and stained of all females, but only the smears of the control group (group A) and the high-concentration group (group D) were evaluated. (Results see Report, Tables 25 - 26, Appendices 21-22)

### *Sperm parameters (Parental animals)*

Parameters examined in all male parental generations:testis weight, epididymis weight, sperm count in testes, sperm count in epididymides, enumeration of cauda epididymal sperm reserve, sperm motility, sperm morphology (Report 4.10.11 / 4.10.13).In addition, the evaluation of homogenization-resistant spermatids was performed in the control group and high-dose group, if no treatment-related changes were

observed in the testes of the high-dose group.

### *Litter observations*

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: yes
- If yes, maximum of 8 pups/litter [4/sex/litter as nearly as possible); excess pups were killed, examined externally for abnormalities and subsequently preserved in a neutral aqueous phosphate buffered 4% solution of formaldehyde.

PARAMETERS EXAMINED
The following parameters were examined in F1 / F2 offspring:number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, weight gain, physical or behavioural abnormalities.G

ROSS EXAMINATION OF DEAD PUPS
:yes, for external and internal abnormalities; dead pups were preserved; possible cause of death was not determined for pups born or found dead (Report 4.10. 12).

### *Postmortem examinations (Parental animals)*

SACRIFICE
- Male animals: All surviving animals were sacrificed after successful mating.
- Maternal animals: All surviving animals were sacrificed at or shortly after weaning on postnatal day 21.

GROSS NECROPSY
- Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

HISTOPATHOLOGY / ORGAN WEIGHTS
The following tissues/organs were prepared for microscopic examination:epididymides, ovaries, pituitary gland, prostate, seminal vesicles and coagulating glands, spleen, testes, uterus, and vaginaFurthermore, reproductive organs of males that failed to sire (did not mate or mated females were not pregnant) and females that were non-mated or non-pregnant, of the low- and mid-dose groups, were microscopically examined.(Report, 4.10.14)

### *Postmortem examinations (Offspring)*

SACRIFICE
- The F1 offspring not selected as parental animals and all F2 offspring were sacrificed at [#?] days of age.
- These animals were subjected to postmortem examinations (macroscopic and/or microscopic examination) as follows:

GROSS NECROPSY
- Gross necropsy consisted of [external and internal examinations including the cervical, thoracic, and abdominal viscera.]

HISTOPATHOLOGY / ORGAN WEIGTHS
The tissues indicated in Table [#] were prepared for microscopic examination and weighed, respectively.

FURTHER NECROPSY and HISTOLOGY
After selection of the pups for the next generation (see Report, 4.10.10 and Appendix 2), from the

remaining pups 1 male and 1 female pup of each litter was subjected to a thorough necropsy. After exsanguination, pups were examined grossly for pathological changes. Special attention was paid to the organs of the reproductive system. Several organs were preserved for potential histology (brain, spleen, thymus, organs with macroscopic abnormalities).

### *Statistics*

The resulting data were analyzed using the methods given below. $P < 0.05$ was considered as the level of significance.

- Fisher's exact probability test: Clinical findings.
- One way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests: Body weight, body weight gain, food consumption and organ weights data.
- Fisher's exact probability test: Number of mated and pregnant females, the number of pregnant females with implants but no pups, females with live pups, females with stillborn pups, live and dead fetuses or pups and the numbers of litters lost entirely.
- Kruskal-Wallis nonparametric analysis of variance and by the Mann-Whitney U test: Pre-coital time (mean number of days), the duration of gestation, the number of corpora lutea and implantation sites, the total number of pups delivered (mean), the mean number of live pups per litter and pre- and post-implantation loss (%)

. - Fisher's exact probability test: Mortality data and data of the pathology of parent animals.

- One-way analysis of variance followed by Dunnett's multiple comparison test: Sperm parameters.or Kruskal-Wallis non parametric analysis of variance and by Mann-Whitney U test.- Fisher's exact test: Estrus cyclicity (number of acyclic animals and number of animals with prolonged estrus period), ANOVA followed by Dunnett's multiple comparison tests (number of estrus cycles per animal) and Kruskal-Wallis nonparametric ANOVA followed by Mann-Whitney U test (length of the longest estrus cycle).
- One way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests: Sexual developmental parameters (preputial separation, vaginal opening and testes descending).

### *Reproductive indices*

The following parameters of reproductive performance were calculated:

* pre-coital time = time between the start of mating and successful copulation
* duration of gestation = time between gestation day 0 and day of delivery
* mating index= (number of females mated/number of females placed with males) x 100
* male fertility index = (number of males that became sire/number of males placed with females) x 100
* female fertility index = (number of pregnant females/number of females placed with males) x 100
* female fecundity index = (number of pregnant females/number of females mated) x 100
* gestation index = (number of females with live pups/number of females pregnant) x 100

### *Offspring viability indices*

The following parameters were calculated:

* live birth index = (number of pups born alive/number of pups born) x 100
* pup mortality day n = (number of dead pups on day n/total number of pups on day n) x 100
* sex ratio day n = (number of live male pups on day n/ number of live pups on day n) x 100
* number of lost implantations = number of implantations sites - number of pups born alive
* post-implantation loss = [(number of implantation sites - number of pups born alive)/number of implantation sites] x 100

### Any other information on materials and methods incl. tables

Si-content analysis and Si-particle detection (Report 4.10.15) A series of organs and tissues of F1-animals were sampled for possible Si-content analysis and Si-particle detection by electron microscopy (EM). Since females were noticed to be more sensitive than males, these analyses were performed with female animals only (except for testis analysis). Blood and tissues were sampled from five rats of each dose group and control by the end of the investigation. Note: The actual analysis of these organs and tissues is outside the scope of this study. The following tissues and organs were included: Oesophagus, stomach, small intestines (duodenum, jejunum, ileum), large intestines (caecum, colon); liver, kidney, urinary bladder, spleen, testis, brain.

## Results and discussions

### Effect levels

| | |
|---|---|
| **Endpoint** | NOAEL |
| **Generation** | other: P, F1, F2 |
| **Sex** | male/female |
| **Effect level** | 1000 mg/kg bw/day |
| **Basis for effect level / Remarks** | based on test materialoverall effects: marternal or paternal constitution and health , reproductive performance and development |

### Observations: parental animals

***Clinical signs (parental animals)***

no effects

***Body weight and food consumption (parental animals)***

no effects

***Test substance intake (parental animals)***

not examined

***Reproductive function: estrous cycle (parental animals)***

no effects

***Reproductive function: sperm measures (parental animals)***

no effects

***Reproductive performance (parental animals)***

no effects

***Organ weights (parental animals)***

no effects

***Gross pathology (parental animals)***
no effects

***Histopathology (parental animals)***
no effects

***Details on results (parental animals)***
Reproductive performance is summarised in Results table 1 and 2 below.

**Observations: offspring**
***Viability (offspring)***
no effects

***Clinical signs (offspring)***
no effects

***Body weight (offspring)***
no effects

***Sexual maturation (offspring)***
no effects

***Organ weights (offspring)***
no effects

***Gross pathology (offspring)***
no effects

***Details on results (offspring)***
Reproductive performance is summarised under "Any other information on results", table 1 and 2 below .

**Remarks on results including tables and figures**

see attachment

**Overall remarks, attachments**

**Overall remarks**

Based on the absence of effects on fertility and developmental parameters, the NOAEL for NM-200 Synthetic Amorphous Silica for fertility and developmental toxicity in this study was 1000 mg/kg bw/d. The NOAEL was the highest dose tested.

## Applicant's summary and conclusion

### Conclusions

In conclusion, in this study, oral administration of NM-200 Synthetic Amorphous Silica up to 1000 mg/kg bw/d had no adverse effect on the reproductive performance of rats or on the growth and development of the offspring into adulthood, examined over two consecutive generations. For male and female animals the No Observed Adverse Effect Level (NOAEL) for maternal and paternal toxicity was 1000 mg/kg bw/d. Based on the absence of effects on fertility and developmental parameters, the NOAEL for NM-200 Synthetic Amorphous Silica for fertility and developmental toxicity in this study was 1000 mg/kg bw/d. The NOAEL was the highest dose tested.

### Executive summary

Procedures: The objective of this reproduction toxicity study (two generation, OECD 416) was to provide data on the possible effects of NM-200 Synthetic Amorphous Silica on reproductive performance of Wistar rats and on the growth and development of the offspring into adulthood after daily oral administration of the test item by gavage for two consecutive generations. Wistar rats of both sexes were administered by gavage a dispersion of the test item in 0.5% methylhydroxypropylcellulose in water. The test material forms agglomerates which had been broken down by stirring of the dispersion as far as possible. After homogenization, the hydrodynamic particle-size distribution was determined in the test dispersions. The mean hydrodynamic diameter of the $SiO_2$particles in the 10 g/l study samples varied between 1076-1664 nm and for the 30 g/l study samples between 876-1216 nm, respectively. The measured size of the 100 g/l study samples appeared to be the smallest (409-703 nm), but due to the high concentration of the particles in the samples, they sedimented and aggregated. Furthermore, multiple scattering occurred in these samples which also influenced the measurements.The dose levels were 0, 100, 300 and 1000 mg NM-200 Synthetic Amorphous Silica/kg body weight for the control, low-, mid- and high-dose groups, respectively. The animals received the test substance during a premating period of 10 weeks, during mating, gestation and lactation until sacrifice.Dams were allowed to raise one litter. At the end of the lactation period, pups were weaned and selected for the next generation. F0- and F1-dams were sacrificed at or shortly after weaning. F0– and F1-males were sacrificed after mating.F1-pups were dosed by gavage at the same dose levels as their parents from postnatal day 21 until sacrifice.
Results: Daily clinical observations during the premating, gestation and lactation period did not reveal any test item related remarkable findings in animals' appearance, general condition or behaviour. Only incidental and/or inconsistent effects on body weights, body weight changes and food consumption were seen. Therefore, it was concluded that in both generations no treatment-related effects were observed on body weights, body weight changes and food consumption. In both generations, no treatment-related effects were observed on estrus cycle parameters of the females animals and on sperm parameters of the male animals.In both generations, no effects of the test item were observed on mating, female fecundity, male- and female fertility and gestation indices. No effects were observed on pre-coital tome and duration of gestation. No test item related effects were observed on the incidences of dams with stillborn pups and dams surviving delivery and on implantation loss. No test item related adverse effects were observed on the mean number of pups delivered, the incidences of liveborn- and stillborn pups, the number of pups lost during the lactation period, the sex ratio, clinical observations and necropsy findings. In both generations, no effects were observed on pup weights and pup weight changes. No statistically significant differences were observed among the various groups in timing of testes descent, preputial separation and vaginal opening. No treatment-related effects were observed at gross examination of the stillborn pups, pups that died during lactation and at macroscopic examination of pups at necropsy at PN day 21. Organ weights of pups and of parental animals of both generations were comparable among the various groups. No treatment-related effects were observed.At necropsy of the parental animals, no test substance-related gross changes were observed in the F0- and F1-generation animals. Microscopic examination did not reveal any test item related findings in the male and female animals of the F0- and F1-generations. For

male and female animals (P, F1 and F2), the overall No Observed Adverse Effect Level (NOAEL) was 1000 mg/kg bw/d, corresponding to the highest dose tested.

# 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