Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

14-Nov-2016

English - Or. English

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

**Cancels & replaces the same document of 02 June 2015**

# DOSSIER ON SILICON DIOXIDE (NM 204)
# - PART 5 -

**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.*

**JT03405321**

**Complete document available on OLIS in its original format**


ENV/JM/MONO(2015)14/PART5
Unclassified
English - Or. English

**OECD Environment, Health and Safety Publications**

**Series on the Safety of Manufactured Nanomaterials**

**No. 51**

# DOSSIER ON SILICON DIOXIDE (NM 204)
# - PART 5 -

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.

**This publication is available electronically, at no charge.**

**For this and many other Environment, Health and Safety publications, consult the OECD's World Wide Web site (www.oecd.org/chemicalsafety/)**

**or contact:**

**OECD Environment Directorate,**
**Environment, Health and Safety Division**
**2 rue André-Pascal**
**75775 Paris Cedex 16**
**France**

**Fax: (33-1) 44 30 61 80**

**E-mail: ehscont@oecd.org**

# PREAMBLE

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

Before launching the Testing Programme, the WPMN first identified a broad list of possible nanomaterials, and the list was later adjusted to a final selection of eleven MNs for testing[2]. This list comprised: i) fullerenes (C60); ii) single-walled carbon nanotubes (SWCNTs); iii) multi-walled carbon nanotubes (MWCNTs); iv) silver nanoparticles; v) titanium dioxide; vi) cerium oxide; vii) zinc oxide; viii) silicon dioxide; ix) dendrimers; x) nanoclays; and xi) gold nanoparticles. One fundamental criterion for selecting these materials was that they should be either in commercial use at the time or expected to be in the near future. At the same time, other considerations were also given attention, such as the production volume of the materials, the likely availability of such materials for testing and the existing information that would readily be available on the materials.

It was also agreed that 59 endpoints would be addressed[3] for each material corresponding to the following categories: i) nanomaterial information/ identification; ii) physical-chemical properties and material characterisation; iii) environmental fate; iv) toxicological and eco-toxicological effects; v) environmental toxicology; vi) mammalian toxicology; and vii) material safety. These endpoints were judged to be most important based largely on the general experience of testing chemicals, while taking into account the potentially different or new properties of nanomaterials. It is worth noticing that it was not expected that testing for all of the listed endpoints would be necessary for each of the selected MNs.

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

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

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

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

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

[ENV/JM/MONO(2009)20/REV][4]. The objective of this Guidance Manual was to guide sponsors[5] in the testing of the materials while ensuring that the information collected was reliable, accurate, consistent and therefore also comparable. The Guidance Manual addressed a whole range of issues including the organisation of the work.

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

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

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

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

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

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

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

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

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

# FOREWORD

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

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

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

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

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

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

[8] http://www.oecd.org/env/testguidelines

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

# ACKNOWLEDGMENTS

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

# TABLE OF CONTENTS

**Name** NM-204 silicon dioxide

## Substance: NM-204 silicon dioxide

# 1. GENERAL INFORMATION

## 1.1 Identification

**Substance identification**

**Chemical name NM-204 silicon dioxide**

## 1.2 Composition

## 1.3 Identifiers

## 1.4 Analytical information

## 1.5 Joint submission

## 1.6 Sponsors

## 1.7 Suppliers

## 1.8 Recipients

## 1.9 Product and process oriented research and development

# 2. CLASSIFICATION AND LABELLING

## 2.1 GHS

## 2.2 DSD - DPD

# 3. MANUFACTURE, USE AND EXPOSURE

## 3.1 Technological process

## 3.2 Estimated quantities

## 3.3 Form in the supply chain

## 3.4 Identified uses and exposure scenarios

## 3.5 Uses advised against

## 3.6 Waste from production and use

## 3.7 Exposure estimates

## 3.8 Biocidal information

## 3.9 Application for authorisation of uses

# 4. PHYSICAL AND CHEMICAL PROPERTIES

## 4.1 Appearance

## 4.2 Melting point/freezing point

## 4.3 Boiling point

## 4.4 Density

## 4.5 Particle size, size distribution

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

#### Administrative Data

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

**Study result type** experimental result

**Data source Reference**

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

#### Data access

other: Owner: NANOGENOTOX

#### Materials and methods

#### Test guideline/method

**Qualifier** equivalent or similar to

**Guideline** other guideline: NIST 960-1 Guideline

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

## Methods

TEM BF-TEM ( Bright Field Transmission Electron Microscopy)

## Principles of method if other than guideline (including performance, 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.o Since these assumptions were not met, the non-parametric Kruskal-Wallis one way ANOVA was performed and data were compared pairwise with Dunn's Method to determine the micrograph and sample effects, and to determine the effect of sonication on the number of particles per grid area.
- The normality of the distributions and the homogeneity of variances were met for the mean values of the median mean diameter.
- A one way analysis of variance (ANOVA) was performed and data were compared pairwise with the Tukey test. The measured parameters were classified by principle component analysis usingo

the SAS statistical software (SAS Institute Inc., Cary, NC, USA).

- Descriptive statistics and histograms were calculated in Sigmaplot (Systat, Cosinus Computing, Drunen, The Netherlands).

***Used Protocols***

1. Dispersion of the sample:NM sample was suspended in double distilled water at a concentration of 2.56 mg/ml and sonicated for 16 minutes using a Vibracell™ 75041 ultrasonifier (750 W, 20kHZ, Fisher Bioblock Scientific, Aalst, Belgium) equipped with a 13 mm horn (CV33) at 40% amplitude. This setup resulted in an average horn power of about 26 W and a sample specific energy of 2530 ± 20 MJ/m³. During sonication the samples were cooled in icy water with ice to prevent excessive heating. After sonication, the samples were diluted to a concentration of 0.512 mg/ml.Details of used procedure can be found in the nanogenotox dispersion protocol file.
2. Grid adjustment: The charge of grid whas adjustes in order to allows for the attachment of the negatively charged silica NM to the EM grid. Alcian blue pretreatment introduced positive charges on the surface of pioloform- and carbon-coated grids that tend to have a negative or neutral charge. (Authors hand experience suggests that this approach is easier than the alternative based on glow discharging EM-grids with air to introduce negative charges and subsequent $Mg^{2+}$ treatment, introducing positive charges). For TEM measurments the suspended NM was brought on pioloform- and carbon- coated, 400 mesh copper grids (Agar Scientific, Essex, England) that were pretreated with 1% Alcian blue (Fluka, Buchs, Schwitzerland). More details about the step by step procedures used for TEM analysis at Coda-Cerva can be found in protocols files.

## Used Protocols: attached files

| | |
|---|---|
| **Attached document** | nanogenotox dispersion protocol.pdf / 777.29 KB (application/pdf): ENV/JM/MONO(2015)14/ANN5 |
| **Remarks** | Dispersion protocol |
| **Attached document** | Coating of dispersed NP in liquid on grids for TEM at CODA.doc / 45 KB (application/msword): ENV/JM/MONO(2015)14/ANN5 |
| **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

???

## Test materials

## Test material equivalent to submission substance identity

yes

## Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

## State of test material

other: fluffy powder

## Overall remarks, attachments

## Attached full study report

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

## Applicant's summary and conclusion

## Conclusions

Primary particle size 19 nm and by manual measurements 10-15 nm.

## Cross-reference to other study

http://www.nanogenotox.eu/

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

### Administrative Data

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

**Study result type** experimental result

### Data source

### Reference

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

### Data access

other: Owner: NANOGENOTOX

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

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

### Materials and methods

### Methods

DLS

**Data gathering**

### Reproducibility

3 measurements

### Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### State of test material

other: fluffy powder in dispersion

## Results and discussions

### Remarks on results including tables and figures

The material is polydisperse.

## Overall remarks, attachments

### Attached full study report

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

## Applicant's summary and conclusion

### Cross-reference to other study

http://www.nanogenotox.eu/

## 4.6 Vapour pressure

## 4.7 N-octanol-water partition coefficient

## 4.8 Water solubility, hydrophilicity, dispersibility

## 4.9 Solubility in organic solvents / fat solubility

## 4.10 Surface tension

## 4.11 Flash point

## 4.12 Auto flammability

## 4.13 Flammability

## 4.14 Explosiveness

## 4.15 Oxidising properties

## 4.16 Oxidation reduction potential

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

## 4.18 Storage stability and reactivity towards container material

## 4.19 Stability: thermal, sunlight, metals

## 4.20 pH

## 4.21 Dissociation constant

## 4.22 Viscosity

## 4.23 Additional physico-chemical information

### *Endpoint study record: composition by DTA by ICM-BAS*

**Administrative Data**

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

**Study result type** experimental result

## Data source

### Reference

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

### Data access

other: owner: NANOGENOTOX

### Data protection claimed

yes, but willing to share

### Materials and methods

### Endpoint investigated

other: composition by DTA

### Details on methods and data evaluation

In DTA, the reference and the sample undergo identical thermal cycles; they are either heated 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 deviate from 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-204

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### Results and discussions

#### Results

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

### Overall remarks, attachments

#### Attached full study report

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

### Applicant's summary and conclusion

#### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: composition by EDS by IMC-BAS*

### Administrative Data

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

**Study result type** experimental result

### Data source

#### Reference

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

#### Data access

other: owner: NANOGENOTOX

### Materials and methods

#### Endpoint investigated

other: composition by EDS

#### Details on methods and data evaluation

EDS is short for Energy-dispersive X-ray spectroscopy and may be available as an extra analytical 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 again during which X-rays are emitted. The emitted X-rays are characteristic for each element and have specific

energetic wavelengths and energy patterns. Therefore an elemental composition can be quantified by analyzing the energy spectrum and intensities of the X-rays emitted during the analysis.EDS is mostly possible for Na and heavier elements. Lighter elements from Be and up may also be quantified depending detectors and instrumental configuration. Oxygen is normally not analysed bySEM EDS, but may be calculated by difference or by converting all elements to oxides. Whencalculated by difference, as done in this work, the sum of all elements adds up to 100 wt%.Measurements may be made as semiquantitative or quantitative analyses using either standardless/internal instrument standard values or calibrated concentration-intensity curves using arange of relevant metals, minerals and glass standards, respectively. In the present analysis, elements were reported as semi-quantitative results. Due to current quality of detectors and in-build standard references, such results are relatively reliable for major elements if the materials have sufficiently high thickness and low roughness.Samples were prepared by pelletizing a known amount of powder. The results are given in wt % and parts per million (ppm) depending on the absolute concentrations in the sample materials.

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### Results and discussions

### Results

Na (ppm/wt%): 1800; Al (ppm/wt%): 4800; Si (wt%): 45.96; S (ppm/wt%): 2100; Ca(ppm/wt%): 0; O (wt%): 53.17

### Remarks on results including tables and figures

no data on the instruments used.

## Overall remarks, attachments

### Attached full study report

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

## Applicant's summary and conclusion

### Conclusions

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

### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: composition by ICP_OES by CODA-CERVA*

### Administrative Data

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

**Study result type** experimental result

### Data source

### Reference

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

### Data access

other: Owner: NANOGENOTOX

### Data protection claimed

yes, but willing to share

### Materials and methods

### Endpoint investigated

other: Elemental composition

### Details on methods and data evaluation

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

### Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### Results and discussions

### Results

Impurities ranges found for NM-204
Impurities > 0.01% : Al, Na > 0.1%, S
Impurities 0.005-0.01 %: Ca
Impurities 0.001-0.005 %: Fe, Zr

### Overall remarks, attachments

### Attached full study report

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

### Applicant's summary and conclusion

### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: composition by TGA by NRCWE*

### Administrative Data

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

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

**Data source**
**Reference**

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

### Data access

other: owner: NANOGENOTOX

## Materials and methods

### Endpoint investigated

other: mass lost by TGA

### Details on methods and data evaluation

In a thermogravimetric measurement a sample is heated in a gas (usually air, $O_2$ or $N_2$) and the weight of the sample is measured as a function of the temperature. The decomposition temperature and loss of mass may give information about the sample, e.g. water adsorbed to the surface of particles will evaporate around 100 °C, whereas most other associated or technically added organic coatings will evaporate or combust at higher temperature. A decomposition in several steps will indicate a non-homogeneous sample containing several different types combustable compounds, which could in fact all be 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 aluminaand had a volume of 70 µL or 150 µL.

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### 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 Publication960-19General descriptionTGA is short for thermogravimetric analysis. The principle is measuring sample weight as a function of temperature in a given atmosphere at a given heating rate.TGA is measured according to information wanted and material investigated. If information about evaporation is wanted heating in $N_2$ is recommended. If information about organic content is wanted heating in $O_2$ or air is recommended, as this will insure combustion of all organic material. In order to make sure e.g. all organic material is decomposed, it is recommended to run to 1000 C.
Materials and Chemicals: Powder (may be conditioned in a specific atmosphere and humidity conditions)
Laboratory weigh (scale)
Apparatus for thermogravimetric analysis
Procedure Sample preparation:
♣ Weigh container.
♣ Fill container with material. Do not stamp it, as this may affect 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 some what 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 th eweight 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/min is not slow enough to avoid spontaneous combustion. There is no spontaneous combustion with a heating rate of 2.5°C/min. The measurement time is very long, app 7 hours per measurement, but this is still recommended. In order to minimize measuring time it may be an option only to heat to9 00 C or even lower.
Data treatment:
Compare TGA curve and curve for first derivative to find steps of weight loss. It is recommended to obtain several measurements to calculate the mean and standard deviation of the weight loss and the evaporation/decomposition temperatures. (the last is most easily found from the curve of the first derivative). The test of multiple samples also enables evaluation of sample homogeneity.

## Results and discussions

### Results

TGA measurements on the NM sample were performed once only as the quantities analyzed were sufficiently large to be representative, and the main purpose for these measurements has been to detect coating on the materials.Weight losses below 100°C for NM-200, suggests that this sample contain 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

## 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

2% mass loss below 100°C (water). Gradual mass loss above 110°C, more than 1% which may indicate organic coating.

### Cross-reference to other study

http://www.nanogenotox.eu/

## 4.24 Agglomeration/aggregation

## 4.25 Crystalline phase

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

#### Administrative Data

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

**Study result type** experimental result

#### Data source

#### Reference

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

#### Data access

other: owner: NANOGENOTOX

#### Data protection claimed

yes, but willing to share

#### Materials and methods

#### Methods

x-ray diffraction (XRD)

#### Principles of method if other than guideline

X-Ray Diffraction (XRD) analysis is based on the principle that crystalline materials diffract X-rays in acharacteristic pattern, which is unique for each material. XRD can therefore be used to identifydifferent 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 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 can be quite difficult to find their actual way of calculation, but they are more or less based on the sameprinciples 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 half maximum).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 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 Advanced diffractometer in reflection mode with Bragg-Brentano geometry. The analysis were made using CuKα1 X-rays (1.5406 Å) generated using a sealed Cu X-ray tube run at 40 kV and 40 mA. The x-ray beam was filtered for CuKα2 and focused using a primary beam Ge monochromator and fixed divergence slit 0.2°. The analyses were made in the stepping mode stepping 0.02 degree 2theta per second and data were collected using a linear PSD detector (Lynx-eye) with opening angle 3.3°.

## Calibration

The analysis were made usin gCuKα1 X-rays (1.5406 Å) generated using a sealed Cu X-ray tube run at 40 kV and 40 mA. The x-ra ybeam was filtered for CuKα2 and focused using a primary beam Ge monochromator and fixed divergence slit 0.2°. The analyses were made in the stepping mode stepping 0.02 degree 2theta per second and data were collected using a linear PSD detector (Lynx-eye) with opening angle 3.3°. Each instrument has a unique contribution to the X-ray diffraction profile, which should be documented for detailed data comparisons using e.g., a large crystallite standard. For the analysis, NRCWE used a $CeO_2$ (NIST SRM674a) standard. To assess the contribution from the instrument,the full width at half maximum, FWHM, was measured on the standard and plotted as a radia nangle.

### Reproducibility

### Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### 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 sample holder. The sample seem to “jump out” of the sample holders with only the slightest disturbance e.g.when using the glass plate to press the samples into the holders. Instead they were mounted with vacuum grease in a single crystal Si low background sample holder.Measurements of empty sample holder with vacuum grease only showed an amorphous signal in theXRD spectrum.The powder samples were mounted by smearing as little vacuum grease as possible on the Si sample holder. Then the powder sample was topped on the sample holder and vacuum grease. The most important disadvantage of this procedure is a small shift of the zero point, as the sample is not entirely in the correct position.

## Results and discussions

### Common name

The NM-204 is amorphous.

## Overall remarks, attachments

### Overall remarks

Estimation of amorphous content based on addition of material is not recommended. It is difficult to ensure an effective mixing and by adding a crystalline material one may shadow the presence 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 under investigation.

### Attached full study report

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

### Applicant's summary and conclusion

### Cross-reference to other study

http://www.nanogenotox.eu/

## 4.26 Crystallite and grain size

## 4.27 Aspect ratio, shape

## 4.28 Specific surface area

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

### Administrative Data

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

**Study result type** experimental result

### Data source

### Reference

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

### Data access

other: owner:NANOGENOTOX

### Materials and methods

### Methods

BET

### Principles of method if other than guideline

Surface area and porosity are important characteristics, in understanding the structure, formation and potential applications of different natural materials. For this reason it is important to determin eand control them accurately. The most widely used technique for estimating surface area is the so called BET method (Brünauer, Emmett and Teller, 1938) [5]. The concept of the theory is anextension 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 inl ayers 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 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).Sample preparationno 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 may vary from sample to sample due to difference in density etc.). Details of the method and values of used paramters might be found in the attached file with full study report : Draft D4.4_specific surface area

### Data gathering

### Instruments

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

### Reproducibility

two measurements were performed for NM-203

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### State of test material

other: fluffy powder

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

The results from the BET analyses conducted in the project were compared with manufacturers data.BET (manufacturer) (m2/g): 140

### Results and discussions

### Specific surface area

**Mean** 136.6 m²/g

**Standard deviation**

### Remarks on results including tables and figures

Total pore volume (mL/g): 0.5057Micropore volume (mL/g): 0.00666

### Overall remarks, attachments

### Attached full study report

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

### Applicant's summary and conclusion

### Conclusions

see the endpoint: compraison between BET and SAXS

### Cross-reference to other study

http://www.nanogenotox.eu/

## *Endpoint study record: Specific surface area by SAXS_CEA*

### Administrative Data

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

**Study result type** experimental result

### Data source

### Reference

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

### Data access

other: owner: NANOGENOTOX

### Data protection claimed

yes, but willing to share

## Materials and methods

### Methods

other: SAXS and USAXS

### Principles of method if other than guideline

Details of the method can be found in the attached SOP document.

### Details on methods and data evaluation

1) Sample preparation: powder samples were prepared in 1.5 mm glass capillaries leading to typical equivalent thickness of dense material from 100 to 200 µm. The usual thickness of aqueous samples for SAXS measurement is 1mm with an acquisition time of 1 hour.Dispersions for analysis are typically produced by sonication in a dispersion medium (see each dedicated SOP (general SOP from NANOGENOTOX) for specific dispersion protocols). The concentration required for analysis depends on the relative scattering length densities between particles and dispersion medium, and the density of materials. The sample must be stable within the time-frame of the measurement.Typical concentration in oxide for NANOGENOTOX suspensions is 3 g/L. Since the scattering length density of silica is relatively low, higher concentrations were used when possible.
2) Details on methodVery detailed description of the method could be found in the attached SOP document.In order to calculate the sample transmission, the flux of incident and transmitted beam are measured and averaged over 200 s before running the SAXS measurement. The time of acquisition necessary for SAXS experiment depends on the sample properties. For $SiO_2$ powders, two measurements were performed: one with a short time of 200 s or 150 s to get unsaturated data for small angles (low q), and one for a long time of 1800 s to get data in the high q region with low signal/noise ratio. For aqueous suspensions prepared for NANOGENOTOX, SAXS measurements were performed in kapton capillaries of internal thickness 1.425 mm and run for 3600s, leading to transmissions of about 0.25. USAXS measurements were performed in 1 mm or 1.5 mm non-sticky double kapton cells.
3) Raw Data Treatment-Raw data, translated into intensity as a function of the scattering vector q, are first normalized by parameters of the experiments such as acquisition time, sample thickness and calibration constants determined using reference samples.
   -The data are thus expressed in absolute scale ($cm^{-1}$).
   -Backgrounds are then subtracted.
   -SAXS data obtained for short time and long time are concatenated, together with USAXS data to get continuous diffractograms on the whole q range.
   -For powder samples, the Porod law is applied to extract specific surface areas of raw materials.
   -Data from suspensions are fitted with a model describing fractal aggregates of primary particles. In this model, the whole q range is divided into sections reflecting different structural levels in the sample, and fitted by local Porod and Guinier scattering regimes.
   -Intensity average parameters are then determined such as radius of gyration for the primaries and for the aggregates, and a fractal dimension for the aggregates.
   -Invariants are calculated, which give a correlation between the sample concentration and the specific surface area obtained in suspension.
4) SSA from SAXS Specific surface area determination from SAXS on powders: To treat raw SAXS data and get absolute intensities, the intensity by the thickness of the scattering material need to be normalised. However, for powder samples, the sample thickness is not well defined and cannot be precisely controlled as it depends on the powder compaction and the different scales of porosity. To elude this problem, a model system is used, considering the effective thickness of material crossed by X-rays, called eB, corresponding to an equivalent thickness if all the material would be arranged in a fully dense (no inner or outer porosity) and uniform layer.Details of the method can be found in the attached file with SOP.

### *Used Protocols*

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

## Used Protocols: attached files

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

**Remarks** Protocol for SAXS measurments in CEA laboratories

## Data gathering

### Instruments

The main set up components used for SAXS and USAXS experiments at CEA/LIONS:

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

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

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

♣ Flux measurement for USAXS set up : DonPhysik ionization chamber

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

♣ Detector for USAXS set up: 1D high count rate CyberStar X200 associated to a scintillator/ photomultiplier detector. All experimental parameters are monitored by computer by a centralized control-command system based on TANGO, and interfaced by Python programming. 2D images are treated using the software ImageJ supplemented with some specific plugging developed at CEA/LIONS. This control-command system has been achieved by Olivier Taché and is detailed in: O. Taché ; « Une architecture pour un système évolutif de contrôle commande d'expériences de physique », Engineer thesis, 2006, available at http://iramis.cea.fr/sis2m/lions/tango/tango-ds/memoire.pdf

### Calibration

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

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### State of test material

other: fluffy powder

### Results and discussions

### Specific surface area

**Mean** 132 m²/g

**Standard deviation** 23 m²/g

### 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 NM200

### Overall remarks, attachments

### Attached background material

**Attached document** SAXS and USAXS for NM200.doc / 157.5 KB (application/msword): SIAR

**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

### Cross-reference to other study

http://www.nanogenotox.eu/

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

### Administrative Data

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

**Study result type** experimental result

### Data source

### Data access

other: owner: NANOGENOTOX

### Materials and methods

### Principles of method if other than guideline

see the conclusions

### 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-204

### Applicant's summary and conclusion

### Conclusions

Comparision between SAXS and BET results.The results from both analytical methods show little difference for NM-204 material.BET specific surface area ($m^2/g$): 136.60. SAXS specific surface area ($m^2/g$): 132.23. Assessed from the methodology, most of the differences may be explained by the combined errors in density and placement of plateau. Other explanations may come from the difference in thermal treatment and outgassing of the powders before BET analysis. 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 $Å^{-1}$, whichcorresponds 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 NANOGENOTOX data. This suggests that producer instrumental capacity and the SOPs for making BET analysis are similar or of same quality as the procedures used in NANOGENOTOX. All being well as SAXS datac onfirms the obtained results.

## 4.29 Zeta potential

## 4.30 Surface chemistry

## 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

## Reference

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

## Data access

other: owner: NANOGENOTOX

## Data protection claimed

yes, but willing to share

## Materials and methods

## Methods

other: Small Rotating Drum method

## Principles of method if other than guideline

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

## Details on methods and data evaluation

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

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

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

## Data gathering

### Instruments

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

## Test materials

### Test material equivalent to submission substance identity

yes

### Reference Material/Nanomaterial and Sample identification number

**Identifier** Reference Material/Nanomaterial

**Identity** NM-204

### State of test material

other: fluffy powder

### Results and discussions

### Remarks on results including tables and figures

Test mass (g): 2
Dustiness indexNumber (1/mg) CPC: 8.25E+06
Inhalable (Mass (mg/kg)): 24969(± 601)
Respirable (Mass (mg/kg)): 1058 (± 1)

### Overall remarks, attachments

### Overall remarks

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

### Attached full study report

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

### Applicant's summary and conclusion

### Cross-reference to other study

http://www.nanogenotox.eu/

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

### Administrative Data

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

**Study result type** experimental result

### Data source

### Reference

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

### Data access

other: Owner: NANOGENOTOX

## Data protection claimed

yes, but willing to share

## Materials and methods

### Details on methods and data evaluation

Vortex Shaker (VS) 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 has been performed three times with this setup.
The results of the tests performed with this first version of the VS method leads to the determination of:
- Dustiness indices expressed as the total number of particles emitted (based on data from CPC).
- Particle size-distribution of the aerosol (based on data from ELPITM Classic in its standard configuration).
The CPC used was the Model 3785 Water-based Condensation Particle Counter (TSI, USA). This CPC detects particles from 5 to >3000 nm. It provides a wide, dynamic, particle-concentration range, an essential characteristic for the tests considered. Featuring a single-particle-counting mode with continuous, live-time coincidence correction and a photometric mode, the CPC measures particle number concentrations up to 107 particles/$cm^3$ with high accuracy. ELPI™ (Electrical Low Pressure Impactor) is an instrument to measure airborne particle size distribution and concentration in real-time. It operates in the size range of 7 nm – 10 μm in its standard configuration. Because of its wide particle size range and rapid response (< 5 s), the ELPI™ has been considered an ideal measurement instrument for the analysis of the unstable concentrations and size distributions, or the evolution of size distributions that could be observed in these tests. In order to prevent particle bounce and charge transfer during the tests, all collection substrates used (PVC GELMAN GLA-5000 5μm / 25 mm) have been greased. In the ELPI the measured current signals are converted to (aerodynamic) size distribution using particle size dependent relations describing the properties of the charger, the impact or 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 0.8μm). Fiber backing filters were used to support the polycarbonate filters. Air flow was driven by a personal sampling pump at a flow rate of 1 L/min. The duration of the sampling has been set to 1 hour. The sampling period was set

equal to the duration of a test (1 hour). For some test, the sample was accumulated over two trials in order to have enough particles to observe. Different TEM copper grids having different carbon have been used (Carbon film, Quantifoil Holey Carbon Films or Holey Carbon Support Film). It is important to note that the duration of the test is to be considered as the process is dynamic. In the original INRS protocol developed, the duration of a test was set equal to 3600 s. But in the first version of the set-up as the instruments measure in real time, it is possible to perform the calculation for different durations between 0 and 3600 s. In this report, the calculations based on the CNC data were performed for twodurations: 180 s and 3600 s. The first duration (180 s) was chosen to be consistent with the method SD. For thesecond version of the setup, the duration of the test was set to 3600 s, which corresponds to the originalprotocol of the Vs method. The second version of the setup is used for collecting respirable mass fraction of the emitted aerosol. The respirable mass fraction is obtained by sampling with a GK2.69 cyclone (BGI, UK). The filters have been preweighed and post-weighed following the recommendations of the ISO 15767:2009 on the same analytical balance. Only one test was performed with this setup due to time constraints. This is why the results are not presented with a confidence interval based on reproducibility. However, measurement uncertainty has been calculated for each measurement performed. The dustiness index in respirable mass (mg) of particles per kilogram, was calculated as the respirable mass of generated particles in milligrams divided by the total mass of the test NM sample inkilograms:The recommendations of the standard ISO 15767:2009 were followed to determine the LOD of the weighing procedure for the filters used for sampling respirable mass of particles during this project. The LOD for the PVCGELMAN GLA-5000 (5 μm/37 mm) filters was equal to 20 ng. This value is used to determine the LOD 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-204

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### Results and discussions

### Dustiness Index

**Mean** mg/kg

**Standard deviation**

### Remarks on results including tables and figures

Gravimetric water content and bulk density
Sample mass (mg) 134
Water content (wt % dry) 6%
Bulk density (g/cm$^3$) 0.16
Respirable dustiness index (mg/kg): 14000
Number-based and mass-based dustiness indexes of NM-204
Test mass (mg): 79.4
Dustiness index Time = 180s Number (1/g) CPC (S.D): 1.3E+06 (1.5E+05)
ELPIa (S.D): 2.6E+06 (3.9E+05) Time=3600s CPC (S.D): 3.0E+06 (5.2E+05)
Respirable (S.D): 1.4E+04 (1.22E-02)
S.D=standard deviation calculated over 3 repeats

## Overall remarks, attachments

### Attached full study report

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

## Applicant's summary and conclusion

### Conclusions

Within this project two methods for characterizing the dustiness of nanomaterials in powder have been developed Small Rotatting Drum method and Vortex Shaker Method. The results of the present work suggest that:
- There are different dust generation rate time profiles. This difference in the dynamic of dust generation is reflected in the difference dustiness indices that are calculated.
- Both SD and VS methods gave reproducible results in terms of amount and size distribution of the generated particles for the NM samples in the project.
- All size distributions of as measured by the SD method were bi- or multimodal.
- Airborne particles generated during these tests are agglomerates/aggregates as shown by the few EM observations made on three selected NM. These results are in agreement with those of the existing literature.
- The comparison between the small drum and Vortex shaker shows that no significant correlation between the two can be found. Further evaluation of this method is needed in order to link it the standardized rotatin gdrum method. Dustiness as quantified by particle number or by mass-based dustiness index had for both methods a large range. These findings suggest a corresponding large difference in exposure potential. It is however difficult to say more to the extent the relationship between index Dustiness and actual exposure is not known. The comparison between the small drum and Vortex shaker shows that no significant correlation between the two can be found. Further evaluation of this method is needed in order to link it the standardized rotatingdrum 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 alreadyintegrated into various European research programs to be launched soon. One of them will be realized withinthe framework of the Mandate 461. Dustiness data obtained within this project can therefore contribute with information on the potentiale xposure 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.

### Cross-reference to other study

http://www.nanogenotox.eu/

## 4.32 Porosity

### *Endpoint study record: Porosity by BET by IMC-BAS*

### Administrative Data

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

**Study result type** experimental result

### Data source

### Reference

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

### Data access

other: owner: NANOGENOTOX

### Data protection claimed

yes, but willing to share

### Materials and methods

### Methods

BET

### Principles of method if other than guideline

Surface area and porosity are important characteristics, in understanding the structure, formation and potential applications of different natural materials. For this reason it is important to determine and control them accurately. The most widely used technique for estimating surface area is the so called BET method (Brünauer, Emmett and Teller, 1938) [5].

The concept of the theory is an extension of the Langmuir theory, which is a theory for monolayer molecular adsorption, to multilayer adsorption with the following hypotheses:

a. gas molecules physically adsorb on a solid in layers infinitely;

b. there is no interaction between each adsorption layer; and
c. the Langmuir theory can be applied to each layer.

### Details on methods and data evaluation

BET analyzer operates by measuring the quantity of gas adsorbed onto or desorbed from a solid surface at some equilibrium vapor pressure. The data are obtained by admitting or removing a known quantity of adsorbate gas (Nitrogen) into or out of a sample cell containing the solid adsorbent maintained at a constant temperature below the critical temperature of the adsorbate (at temperature of liquid Nitrogen). As adsorption or desorption occurs the pressure in the sample cell changes until equilibrium is established. The quantity of gas adsorbed or desorbed at the equilibrium pressure is the difference between the amount of gas admitted or removed and theamount 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-204

### Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

### State of test material

other: fluffy powder

## Results and discussions

### Remarks on results including tables and figures

total pore volume (ml/g): 0.5057
micropore volume (ml/g): 0.00666

## Overall remarks, attachments

### Attached full study report

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

**Applicant's summary and conclusion**

**Cross-reference to other study**

http://www.nanogenotox.eu/

# 5. ENVIRONMENTAL FATE AND PATHWAYS

# 6. ECOTOXICOLOGICAL INFORMATION

# 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-204.

### 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-204.

## 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.

## Applicant's summary and conclusion

### Interpretation of results

no bioaccumulation potential based on study results

# 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** 21 Aug. - 04 Sep. 1990

**Reliability** 1 (reliable without restriction)

**Rationale for reliability** GLP guideline study

#### Data source

#### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Jahn W, Zechel H-J, Berthold K | **Year** | 1977 |
| **Title** | Testing the acute toxicity after single oral administration in rats | | |
| **Bibliographic source** | unpublished report | | |
| **Testing laboratory** | ASTA Pharma AG | **Report no.** | 878894 |
| **Owner company** | Evonik Industries AG | | |
| **Company study no.** | Degussa AG No. US-IT-90-0038-DGT | **Report date** | 1990-11-16 |

#### 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** 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-204.

### Details on test material

>98% ($SiO_2$), $Na_2O$ <1%, $Al_2O_3$ <0.2%, $SO_3$ <0.8%,$Fe_2O_3$ <0.03%: CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8

### Confidential details on test material

The test substance is equivalent to NM-204.

## Test animals

### Species

rat

**Strain**

Wistar

**Sex**

male/female

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

TEST ANIMALS
- Source: Winkelmann Versuchstierzucht, Borchen/Germany
- Age at study initiation: 9 wks (male), 10 wks (females)
- Weight at study initiation: 183 - 191 g (male), 141 - 152 g (female)
- Fasting period before study: 16 h before start
- Housing: single in Macrolon cages
- Water: ad libitum
- Acclimation period: ≥ 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20.5 - 22.5 °C
- Humidity (%): 40 - 70 %
- Photoperiod (hrs dark / hrs light): 12 / 12 hours

## Administration / exposure

### Route of administration

oral: gavage

### Vehicle

other: aqueous suspension with 1 % carboxymethyl cellulose

***Details on oral exposure***

VEHICLE
- Concentration in vehicle: 237 mg silica/mL suspension
- Amount of vehicle (if gavage): 21.5 ml/kg bw (including 5100 mg TS)
- Justification for choice of vehicle: suspending the test material and stabilising the suspension

MAXIMUM DOSE VOLUME APPLIED: 21.5 ml/kg bw

### Doses

5110 mg/kg bw237 mg/ml

### 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: days 0, 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

5 male and 5 female animals were used. The dose was applied by gavage as aqueous suspension (21.5 ml/kg bw = 237 mg silica/ml suspension) containing 1 % CMC.

## 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 weight gain

***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** | 1978 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability** | Comparable to guideline study with acceptable restrictions | | |

### Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Woltjen R, Calkins JE | **Year** | 1978 |
| **Title** | Acute oral LD50 in the rats | | |
| **Bibliographic source** | unpublished report | | |
| **Testing laboratory** | Huntingdon Research Center (HRC) | **Report no.** | HRC #N 783-212 HRC #N 783-215 HRC #N 783-218 HRC #N 783-221 |
| **Owner company** | J.M. Huber Corporation | | |
| **Company study no.** | | **Report date** | 1978-03-23 |

### 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** 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-204.

### Details on test material

CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8

### Confidential details on test material

The test substance is equivalent to NM-204.

### Test animals

### Species

rat

### Strain

Sprague-Dawley

### Sex

male/female

## Administration / exposure

### Route of administration

oral: gavage

### Vehicle

water

### Doses

10000, 12600, 15800, and 20000 mg/kg

### No. of animals per sex per dose

5

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

Five animals per sex and group were used. Substances were suspended in water, administration by gavage. Observation period 14 days.

## Results and discussions

### Effect levels

**Sex** male/female

**Endpoint** LD0

**Effect level** > 20000 mg/kg bw

**95% CL**

**Remarks**

#### *Mortality*

none

### Remarks on results including tables and figures

No clinical symptoms; after 1 day, the stools were white coloured (reversible after 2 days).

## Overall remarks, attachments

### Overall remarks

Method: suspended in water (33 % w/w); administration by gavage. Observation period 14 days. Results: no clinical symptoms; after 1 day the stools were white coloured (reversible after 2 days)

## 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-204.

### 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
Primary particle size: see Test Condition

### Confidential details on test material

The test substance is equivalent to NM-204.

### 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 (µm)** |
|---|---|
| 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 abouit 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

## 7.2.3 Acute toxicity: dermal

## *Endpoint study record: Acute toxicity: dermal.001*

**Administrative Data**

| | | | |
|---|---|---|---|
| **Purpose flag** | weight of evidence (X) robust study summary ( ) used for classification ( ) used for MSDS | | |
| **Study result type** | experimental result | **Study period** | 1978 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability** | Comparable to guideline study with acceptable restrictions | | |

## Data source

## Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Woltjen R, Calkins JE | **Year** | 1978 |
| **Title** | Acute dermal LD50 in the rabbit | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | Huntingdon Research Center (HRC) | **Report no.** | HRC #N 783-213 HRC #N 783-216 HRC #N 783-219 HRC #N 783-222 |
| **Owner company** | J.M. Huber Corporation | | |
| **Company study no.** | | **Report date** | 1978-04-26 |

## 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** no guideline available

**Guideline**

**Deviations**

## Principles of method if other than guideline

"Standard acute method under occlusive conditions"

## GLP compliance

no

## Test materials

## Test material equivalent to submission substance identity

yes

## Test material identity

**Identifier** CAS number

**Identity** 7631-86-9

**Identifier** EC number

**Identity** 231-545-4

**Identifier** IUPAC name

**Identity** dioxosilane

**Details on test material**

CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8

**Test animals**

**Species**

rabbit

**Strain**

New Zealand White

**Sex**

no data

**Administration / exposure**

**Type of coverage**

occlusive

**Vehicle**

water

***Details on dermal exposure***

Four animals per group used, two each treated on the intact and abraded skin: The substance was mixed with distilled water to form an aqueous paste

**Duration of exposure**

24 h

**Doses**

2000, 3000, 4000, and 5000 mg/kg

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

4 per dose group

***Details on study design***

- Duration of observation period following administration: 14 days
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, body weight, organ weights

**Results and discussions**

**Effect levels**

**Sex**

**Endpoint** LD50

**Effect level** > 5000 mg/kg bw

**95% CL**

**Remarks**

### Remarks on results including tables and figures

Local effect: very slight erythema (score 1 of 4), reversible after 2 days or 5 d in one or a few animals. No systemic signs of toxicity or organ toxicity.

### Applicant's summary and conclusion

### Interpretation of results

other: non-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** 09 - 19 Oct. 1990

**Reliability** 1 (reliable without restriction)

**Rationale for reliability** GLP guideline study

### Data source

### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Jahn W, Berthold K | **Year** | 1991 |
| **Title** | Testing the primary irritation/corrosion after single application to the skin of the rabbit | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | ASTA Pharma AG | **Report no.** | 878905 |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 91-0131-DGT | **Report date** | 1991-01-18 |

### 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** OECD Guideline 404 (Acute Dermal Irritation / Corrosion)

**Deviations**

### 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-204.

### Details on test material

>98% ($SiO_2$), $Na_20$ <1%, $Al_2O_3$ <0.2%, $SO_3$ <0.8%,$Fe_2O_3$ <0.03%: CAS
-Name: Silica, precipitated, cryst.-free;
CAS-No.: 112926-00-8

### Confidential details on test material

The test substance is equivalent to NM-204.

### Test animals

### Species

rabbit

### Strain

other: White Russian

### Test system

### Type of coverage

occlusive

### Preparation of test site

shaved

### Vehicle

water

### *Amount/concentration applied*

Dose: 0.5 g

### Duration of treatment / exposure

24 hour(s)

### Observation period

14 days

### Number of animals

3

### Control animals

no

### *Details on study design*

The substance (0.5 g) was moistened with 0.5 ml water and placed on a skin area of approx 6.25 $cm^2$.

## Results and discussions

### Irritation / corrosion results

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

**Basis**

**Time point**

**Score** 0

**Max. score**

**Reversibility**

**Remarks**

### *Irritant/corrosive response data*

There were not any irritating effects.

### Remarks on results including tables and figures

The single application (4hours, occlusive patch) of 0,5g test substance to the intact skin of three rabbits each caused no changes. During the observation period neither erythema nor endema could be detected. The irritation index is 0.0. The test substance therefore is classified as non-irritant in this test system.

## Overall remarks, attachments

### Overall remarks

The irritation index is 0.0.Test substance therefore is classified as non-irritant in this test system.

### 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** 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 détermination de l´indice d'irritation 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-204.

### 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-204.

## 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 mg
Volume: 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)

## 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** 22 Oct. - 05 Nov 1990

**Reliability** 1 (reliable without restriction)

**Rationale for reliability** GLP guideline study

#### Data source

#### Reference

| | | | |
|---|---|---|---|
| **Reference type** | study report | | |
| **Author** | Jahn W and Berthold K | **Year** | 1991 |
| **Title** | Testing of the primary irritation after single application to the eye of the rabbit | | |
| **Bibliographic source** | Unpublished report | | |
| **Testing laboratory** | ASTA Pharma AG | **Report no.** | 878916 |
| **Owner company** | Evonik Degussa GmbH | | |
| **Company study no.** | Degussa AG - US-IT-No. 91-0132-DGT | **Report date** | 1991-01-16 |

#### 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** OECD Guideline 405 (Acute Eye Irritation / Corrosion)

**Deviations**

#### 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-204.

### Details on test material

CAS-Name: Silica, precipitated, cryst.-free; CAS-No.: 112926-00-8
$SiO_2$ >98% , $Na_20$ <1%, $Al_2O_3$ <0.2%, $SO_3$ <0.8%,$Fe_2O_3$ <0.03%:

### Confidential details on test material

The test substance is equivalent to NM-204.

## Test animals

### Species

rabbit

### Strain

other: White Russian

## Test system

### Vehicle

unchanged (no vehicle)

***Amount/concentration applied***

Dose: 100 mg

### Duration of treatment / exposure

24 h, not rinsed

### Observation period

7 days

### Number of animals

3

### Control animals

not required

***Details on study design***

Comment: not rinsed

REMOVAL OF TEST SUBSTANCE
- Washing (if done): not rinsed
- Time after start of exposure: 24 h

SCORING SYSTEM:
Intensities of irritation effects by using the Draize scale for grading of lesions (scores 0 - 4).
Irritation index: calculated according to Draize from grades on cornea, iris and conjunctiva at the time 1, 24, 48 and 72 h,modified according to Gilman et al. 1983

**Results and discussions**

***Irritant/corrosive response data***

There were weakly irritating effects on the conjunctivae only: redness score 2 (of 4) in all animals after 1 h, score 2 and 1 after 24 h and reversible by 72 h.
Chemosis and discharge was very slight only 1 h after application (score 1).

**Remarks on results including tables and figures**

There were weakly irritating effects on the conjunctivae only: redness score 2 (of 4) in all animals after 1 h, score 2 and 1 after 24 h and reversible by 72 h.
Chemosis and discharge was very slight only 1 h after application (score 1).

**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 | **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.** | V81.268/20174 1 |
| **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-204.

### 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-204.

### 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 %
Mean estimated doses: 300-330, 1200-1400, 4000-4500 mg

**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: NoHAEMATOLOGY: 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-204.

### Details on test material

CAS-Name: Silica, precipitated, crystalline-free; CAS-No.: 112926-00-8
Surface area (Ströhlein): 160 - 195 $m^2$/gPrimary 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 $A_{l2}O_3$
- Stability under test conditions: stable
- Storage condition of test material: room temperature

### Confidential details on test material

The test substance is equivalent to NM-204.

## 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/m3 (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 g10 each (sacrificed after 13 wks), ù50 each kept for a recovery period of at most 52 wks (13, 26, 39, and 52 wks).

## Control animals

yes

### *Details on study design*

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

### *Positive control*

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

## Examinations

### *Observations and examinations performed and frequency*

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

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

BODY WEIGHT: Yes
- Time schedule for examinations: start, weekly during exposure, 1x/wk during recovery
- 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
- Time schedule for examinations:

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: Yes
- Time schedule for collection of blood: week 13, 26, 39, 52, 65 (i.e. including recovery period)
- Anaesthetic used for blood collection: No (data)
- Animals fasted: No data
- How many animals: 10 males, 10 females
- 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 nodes
in 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 test
Organ 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 males and 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-204.

### 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-204.

**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/m3) [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/m3 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 CONTENT
Si 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 /** | Histopathology: based on the pulmonary response (inflammation reaction) (see details below "Details on results") |

**Remarks**

| | |
|---|---|
| **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 month (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 infiltrates, 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$^3$.