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

08-Jun-2016

English - Or. English

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

SILICON DIOXIDE: SUMMARY OF THE DOSSIER

Series on the Safety of Manufactured Nanomaterials
No. 71

This document is only available in PDF format.
This document is the summary of the Silicon dioxide dossier published as:
- ENV/JM/MONO(2015)14/PART1
- ENV/JM/MONO(2015)14/PART2
- ENV/JM/MONO(2015)14/PART3
- ENV/JM/MONO(2015)14/PART4
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JT03397644

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ENV/JM/MONO(2016)23
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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 71

SILICON DIOXIDE: SUMMARY OF THE DOSSIER

IOMC
INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
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Environment Directorate
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Paris, 2016

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Nos. 44-54, These items are the dossiers derived from the Testing Programme on Manufactured Nanomaterials which are located at:
http://www.oecd.org/chemicalsafety/nanosafety/testing-programme-manufactured-nanomaterials.htm

No.55, *Harmonized Tiered Approach to Measure and Assess the Potential Exposure to Airbone Emissions of Engineered Nano-objects and their Agglomerates and Aggregates at Workplaces. (2015)*

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No.62, *Considerations for using dissolution as a function of surface chemistry to Evaluate environmental behaviour of nanomaterials in risk assessments (2015)*

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No.66, *Categorisation of manufactured nanomaterials (2016)*

No.67, *Developments in delegations on the safety of manufactured nanomaterials - tour de table(2016)*

No.68, This document is the summary of the dossier Multi-walled Carbon Nanotubes (MWCNT) derived from the Testing Programme on Manufactured Nanomaterials which are located at: http://www.oecd.org/chemicalsafety/nanosafety/testing-programme-manufactured-nanomaterials.htm

ABOUT THE OECD

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

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

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

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

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OVERVIEW

A list of abbreviations is available as appendix VII.

1. **Chemical Name:** Silicon Dioxide, Synthetic Amorphous
2. **CAS Number:** Silicon dioxide, general CAS number: 7631-86-9

   Precipitated silica, CAS number: 112926-00-8 (NM-200, NM-201 and NM-204)

   Pyrogenic/thermal silica, CAS number: 112945-52-5 (NM-202, NM-203)

   EINECS number: 231-545-4
3. **Lead Sponsor(s):** France and the European Commission
4. **Co-Sponsors:** BIAC, Korea, Canada, Belgium and Denmark
5. **Overview of Lead Sponsor(s) and Co-Sponsors:**

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6. **Date of Submission:** Initial submission: 01 Sep. 2013. Date of publication: February 2016
7. **Comments:**

7.a End-points in the WPMN test programme:

The OECD Working Party on Manufactured Nanomaterials (WPMN) testing programme agreed on relevant end-points, see Appendix 0, for the phase 1 of the WPMN testing programme, and this dossier on Synthetic

Amorphous Silicon dioxide (SAS) provides an overview of the outcomes of the testing as well as background information and including a review of relevant literature. Detailed information on results is presented in the appendices to this report. Information about the tests performed is presented in the Annexes 1 to 10, which are published separately to this report.

According to definitions of nanomaterials, for example by ISO, SAS is a nanostructured material. The SAS industry, as co-sponsor, provided several samples of SAS to the lead sponsors, to enable selection of the most relevant sample for the Testing Programme. BIAC furthermore initiated the Cefic Long-Range Research Initiative (LRI) N1 and Cefic LRI N3 programme, see http://cefic-lri.org/, in which NM-200 was tested. When the test results became available, their robust study summaries were provided as an addendum to the WPMN SAS dossier.

The European Joint Action "NANOGENOTOX", which active from 2009 to 2013 and co-financed by the European Commission's Directorate General for Health and Consumers (DG SANCO) and several EU Member States, has contributed significantly to this document by making available all its scientific results on characterisation and mammalian toxicology of SAS. Appendix IV lists the associated and collaborating partners and the outcomes of the project are presented at http://nanogenotox.eu/.

7.b Previous review reports on amorphous silicon dioxide and silicates:

The properties of synthetic amorphous silica has already been reviewed and assessed in several contexts, and the three main review reports identified are:

1) "Synthetic Amorphous Silica and Silicates" by the UK under the OECD High Production Volume (HPV) program. The associated SAS HPV dossier was agreed at SIAM 19, 19-22 October 2004 and published by UNEP [OECD (2004a)].
2) Furthermore, the European Centre for Ecotoxicology and Toxicology of Chemicals, ECETOC, has assessed SAS and published the outcome in JACC report number 51 of September 2006 (ECETOC (2006)).
3) The Synthetic Amorphous Silica and Silicates Industry Association (SASSI) prepared a SAS Voluntary Submittal Package (25 July 2008) for the Nanoscale Materials Stewardship Program (NMSP) of the U.S. Environmental Protection Agency (SASSI (2008)).

Furthermore, a Screening Information Dataset (SIDS) report on soluble silicates was identified (OECD 2004b). Parts of this report on soluble silicates are relevant for SAS, as it states about environmental monitoring data that "Dissolved silica from commercial soluble silicates is indistinguishable from natural dissolved silica. … Compounds of silicon and oxygen are ubiquitous in the environment; they are present in inorganic matter, like minerals and soils as well as in organic matter, like plants, animals and man. Silica is found in all natural waters with an average concentration of 10-20 mg SiO_2/l."

In addition to these reports, the Association of Synthetic Amorphous Silica Producers (ASASP) stated at the meeting on 30th September 2009 that SAS has been registered under REACH. The European Chemicals Agency (ECHA) has published the registration and information is available from ECHA at http://echa.europa.eu; the registration covers synthetic amorphous SiO_2 (SAS) only.

The reports listed above give a comprehensive review of synthetic amorphous silica obtained by different processes and as placed on the market, and contain much valuable information on the material. The

reports reflect that SAS is not a newly developed nanostructured material, but has been placed on the market for decades. Nevertheless, analysing the information presented in the reports in relation to the base data set agreed for a principal material for dossiers in the WPMN sponsorship programme as described in the guidance manual for sponsors (OECD 2010), the data presented in the reports has some limitations. For example, the data relate to different sources of SAS[1], which differ across several physical-chemical properties, depending on the manufacturing process and exact process parameters within each process. The differences include for example the size of primary particles and specific surface area. Furthermore, the reports present only limited data on the physical-chemical characterisation of the different SASs tested and are thus not fulfilling the information requests in the Sponsorship Program. Under the WPMN Testing Programme, validated standard test methods should be used, e.g. OECD, DIN, ISO, or adapted as exemplified in the reports on test methods, nanomaterials and sample preparation (OECD 2009 and OECD 20012) and the agreed data set of 59 end-points should be submitted for one source material. The reports listed above therefore have a limited value for addressing the information needs of the Sponsorship Programme.

In addition to those reports, several scientific articles reporting outcomes of tests using specific sources of SAS have been identified, and this report gives an overview also of these articles grouped according to end-points. The literature survey performed was general and included searches aimed at SAS in general, not one specific source of SAS.

[1] SAS from different producers, obtained through different chemical processes, and with different particle-sizes.

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1. GENERAL INFORMATION

1.1. Substance Information

CAS Number:	Silicon dioxide, general CAS number: 7631-86-9 Precipitated silica (NM-200, NM-201 and NM-204), CAS number: 112926-00-8 Pyrogenic silica (NM-202 and NM-203), CAS number: 112945-52-5
EINECS Number	231-545-4
OECD Name	Silicon dioxide
IUPAC Name:	Silicon dioxide
Molecular Formula:	SiO_2
Molecular Weight:	60.08 g/mol
Synonyms:	Synthetic amorphous silica, SAS, silica

The principal material in the OECD WPMN test programme is NM-200 which is a precipitated synthetic amorphous silicon dioxide (SAS). The two alternate materials NM-201 and NM-204 are also precipitated materials, whereas the alternate materials NM-202 and NM-203 are both pyrogenic SAS. The materials in the NM series are provided by the JRC Nanomaterials Repository, see http://ihcp.jrc.ec.europa.eu/our_activities/nanotechnology/nanomaterials-repository, that has subsampled representative nanomaterials (Roebben at al. 2013) initially for the OECD WPMN testing programme. The background for material selection is given in Appendix 0 to this report.

In addition, information on silicon dioxides, which are not represented by ASASP, was submitted: Korea submitted information for a laboratory synthesized material and manufactured in Sykgyung AT, and furthermore Japan contributed in vitro test results of nanotek SiO_2.

1.2. Details on Chemicals Category

The spatial arrangement of SiO_2 is formed by strong, directional covalent bonds, and has a well-defined local structure: four oxygen (red) atoms are arrayed at the corners of a tetrahedron around a central silicon atom (yellow), see Figure 1.

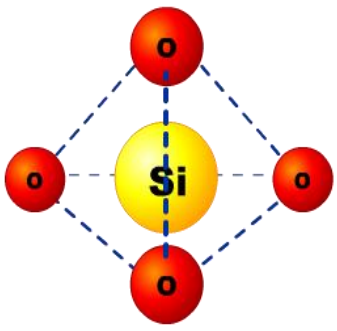


Figure 1. Structure of silicon dioxide.

The bond angles around O-Si-O are essentially the tetrahedral angle, 109 degrees; the Si-O distance is 1.61 A (0.16 nm) with very little variation. The bond angle Si-O-Si, θ_{SiOSi}, is nominally about 147

degrees, but can vary from about 120 to 180 degrees with very little change in bond energy. Furthermore, rotation of the bond about the axis is almost completely free, see Figure 2.

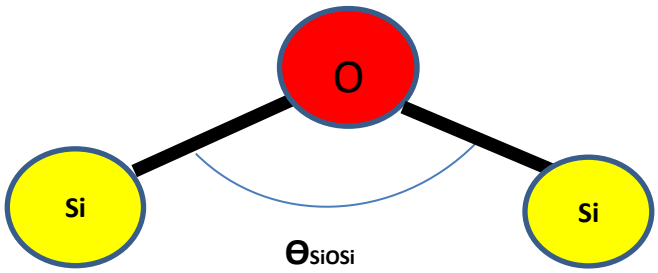


Figure 2. Silicon-Oxygen-Silicon variation of bond angle.

These observations can be summarised as follows: the "tetrahedra" formed by the SiO_4 groups must touch each other at their corners, but can do so at widely varying angles, which is also known as the Zachariesen-Warren model for the structure of SiO_2. The result of this flexibility in the bridge bonds is that SiO_2, while it has many different possible crystalline structures, can very easily form amorphous materials (i.e. materials with no long-range order).

Surface chemistry

At the surface of the SAS NMs two types of groups appear: (1) hydrophobic siloxane, which is oxygen and silicon covalently bound, and (2) hydrophilic silanol (Si-OH), where the oxygen is bound to silicon and hydrogen.

(1) The siloxane groups (Figure 3) are hydrophobic and only slightly reactive functional groups. Siloxane groups could be formed at high temperature during a process of silica dehydration for example through condensation of two silanols:

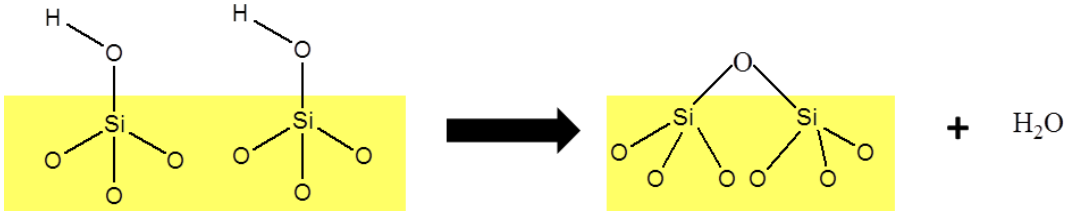


Figure 3: Siloxane group formed by the condensation of two silanol groups

(2) The silanol group may exist in three different spatial arrangements that have different reactivity (Figure 4):

- Isolated group (free silanol) which consist of a silicon atom linked to three bonds in the bulk structure and the fourth one attached to a single OH group (Figure 4A).

- Vicinal group (bridged silanol) where 2 single groups are attached to different silicon atoms and are close enough to form an H-bond (Figure 4B).
- Germinal group which consist of two hydroxyl groups linked to one silicon atom. The germinal groups are too close to form a hydrogen bond, whereas the two groups are too far separated (Figure 4C).

The silanol group is more reactive than the siloxane group. The distribution of these active groups is depending on the silica type, and the method of synthesis. The temperature and the hydration degree are also an important factor. Within the silanol the spatial arrangement, see Figure 4 A, B and C, also influences the reactivity with the Isolated (free) silanol being the most reactive species.

The number of silanol groups per unit surface area (per nm^2) varies from 5.0 to 5.7 for precipitated silica, and from 1.25 to 2.5 for pyrogenic silica. As the silanol group is hydrophilic, the solubility of SAS depends on the number of silanol groups per unit surface area (SASSI 2008, Zhuravlev (2000), and the solubility of silicon dioxide depends also on the route of production. Three possible spatial arrangements have been identified for silanol, see Figure 4.

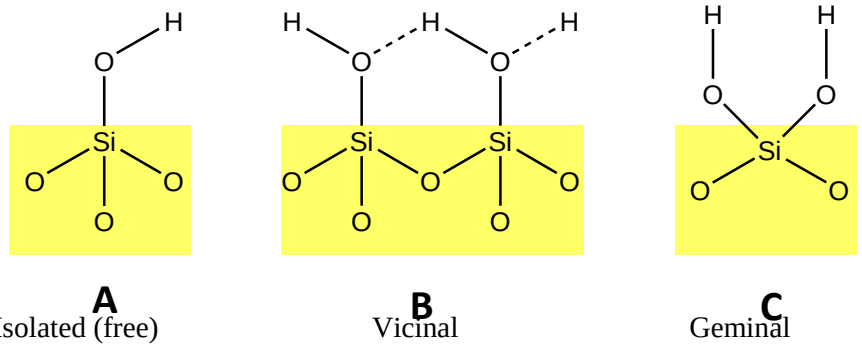


Figure 4. Spatial arrangements for main types of silanol. Isolated (free) silanol (A), Vicinal silanol (B) and germinal silanol (C)

The reactivity of the SAS depends on the degree and the accessibility of the silanol groups, especially the Isolated (free) ones (Zhuravlev (2000). SAS is stable at pH values between 2 and 8, and at a pH above 8 the reactions given below occur at a much higher frequency.

$$(SiO_2)_x + 2\ H_2O \rightleftharpoons (SiO_2)_{x-1} + Si(OH)_4$$

$$2\ Si(OH)_4 \rightleftharpoons H_6Si_2O_7 + H_2O$$

The chemisorption of the hydroxyl group enables release of a monosilicic acid molecule.

Above pH > 9, dissolution of silica increases quickly as silicate ions will be formed from the $Si(OH)_4$ monomer. When catalysed by hydroxide ions (OH^-) this dissolution occurs more rapidly.

$$Si(OH)_4 + OH^- \rightleftharpoons Si(OH)_5^- \rightleftharpoons H_3SiO_4^- + H_2O$$

Characterisation

The principal (NM-200) and alternate materials (NM-201, NM-202, NM-203 and NM-204) were characterised in the NANOGENOTOX project as well as by the European Commission's Joint Research Centre, the JRC. Rasmussen et al. (2013) presents the collected data and information on the physical-chemical characterisation of the NM-series, giving details for both the principal and alternate materials of

the NM-series, and also including an overview of the test methods and procedures. The separate Annexes 1 to 10 of this report provide a description per method of the measument procedures of the Nanogeneotox project for physical chemical characterisation and for genotoxicity testing, as well as results from the testing. Table 1 gives an overview of the physical-chemical characterisation performed and the methods used for the 5 NM-20x tested. Table 3 gives an overview of the results for the principle material, NM-200. Table 4 and Table 5 summarise the characterisation results for all the NM-20x. In addition, Korea submitted information for a laboratory synthesized material and manufactured in Sykgyung AT, and Japan contributed *in vitro* test results of nanotek SiO_2.

Almost all of the OECD endpoints on physical-chemical testing have been completed for the principal OECD WPMN material, NM-200. Also the alternate materials, NM-201, NM-202, NM-203 and NM-204 have been extensively characterised. The determination of the octanol water coefficient (K_{ow}) is not feasible for nanomaterials as discussed at an OECD expert meeting in Mexico, March 2013, see OECD 2014, and was considered to be not relevant for insoluble and sparingly soluble nanomaterials, and K_{ow} has thus not been measured. The photocatalytic activity was considered to be not relevant for SAS and was not measured. Analysis of intrinsic hydroxyl radical formation capacity, using the Benzoic acid probe for quantification, gave no detectable radical after 24 and 48-hour incubation.

In the NANOGENOTOX project a standardised sample preparation protocol was developed and used for the testing; the protocol addressed three types of materials, TiO_2, SiO_2 and MWCNT, and was optimized for the set of materials. Briefly, the final dispersion following the protocol has a concentration of 2.56mg/mL in sterile-filtered 0.05% w/v BSA-ultrapure water. The samples are sonicated (probe sonicator) for 16 minutes, placed in an ice bath, and the energy input should be calibrated to be in the order of 3,136 MJ/m^3. The protocol is available at the project's webpage:
http://www.nanogenotox.eu/files/PDF/web%20nanogenotox%20dispersion%20protocol.pdf

Table 1. SAS NMs: Physical and chemical characterisation performed on the NM-series in the Nanogenotox project and by the JRC (from Rasmussen et al., 2013); for the dispersion protocol see http://www.nanogenotox.eu/. The "x" indicates that the analysis was performed for that material and method combination.

Physical-chemical Properties and Material Characterization (from OECD list)	**NM characterised**					**Method**
	200	201	202	203	204	
Homogeneity	x			x		DLS
Agglomeration / aggregation	x	x	x	x		SAXS/USAXS
	x	x	x	x		DLS
	x	x	x	x		TEM
Water solubility *)	x	x	x	x	x	SDR
Crystalline phase	x	x	x	x	x	XRD
Dustiness	x	x	x	x	x	Small rotating drum
	x	x	x	x	x	Vortex shaker method
Crystallite size	x	x	x	x	x	SAXS/USAXS
	x	x	x	x	x	XRD
Representative TEM picture(s)	x	x	x	x	x	TEM
Particle size distribution	x	x	x	x	x	CLS
	x	x	x	x	x	TEM
	x	x	x	x		DLS
	x	x	x	x	x	AFM
Specific surface area (SSA)	x	x	x x	x x	x x	BET
	x	x	x	x	x	SAXS/USAXS
Volume SSA	x			x		TEM tomography

Zeta potential (surface charge)	x	x	x	x		Zeta-metry
Surface chemistry (where appropriate).	x			x		XPS
Presence of coating (TGA, DTA, GC-MS)	x	x	x	x	x	TGA
	x	x	x	x	x	DTA
					x	GC-MS (TGA_{110} degrees celcius > 1 wt%)
Photo-catalytic activity						
Pour density	x	x	x	x	x	Weighing
Porosity	x	x	x	x	x	BET
Octanol-water partition coefficient, where relevant						
Redox potential	x	x	x	x	x	SDR
OH radical formation, acellular	x	x	x	x	x	Benzoic acid probe to form hydroxybenzoic acid analysed by HPLC-UV
Other relevant information (where available)						
Elemental analysis/impurities	x	x	x	x	x	Semiquantitaive ICP-OES
	x	x	x	x	x	Semiquantitaive EDS

) the solubility was investigated in Gambles solution, Caco 2 medium, and the NANOGENOTOX dispersion medium

An overview of the characterisation data provided by BIAC for the dossier on SAS is given below in Table 2. The actual measurements were performed at various institutes, e.g. Laser diffraction was performed by TU Dresden, Germany.

Table 2. Physical-Chemical Characterisation data provided by BIAC. The "x" indicates that the analysis was performed for that material and method combination.

Physical-chemical Properties and Material Characterization (from OECD list)	**NM characterised**					**Method**
	200	201	202	203	204	
Homogeneity						
Agglomeration / aggregation	x	x	x	x		Laser diffraction spectrometry, ISO 9276-2
	x	x	x	x		DLS
Water solubility *)	x	x	x	x		Motomizu (test substance equivalent to NM-200, NM-201) OECD TG 105
Crystalline phase	x	x	x	x		XRD
Dustiness						
Crystallite size						
Representative TEM picture(s)						
Particle size distribution	x	x	x	x		Laser diffraction spectrometry, ISO 9276-2
	x	x	x	x		DLS
	x	x	x	x		TEM
Specific surface area (SSA)			x			BET
Volume SSA						
Zeta potential (surface charge)			x			Based on laser Doppler anemometry
Surface chemistry (where appropriate). Presence of coating						

Photo-catalytic activity						
Pour density						
Porosity						
Octanol-water partition coefficient, where relevant						
Redox potential						
OH radical formation, acellular						
Other relevant information (where available) Tapped density	x	x	x	x		

1.3. General Substance Information

A. Type of Substance

Element [ ]

Inorganic [X]

Natural substance [ ]

Organic [ ]

Organometallic [ ]

Petroleum product [ ]

B. Physical State (at 20°C and 1.013 hPa)

Gaseous [ ] Liquid [ ] **Solid [X]**

C. Purity (Indicate the percentage by weight/weight)

Different analyses were performed for identifying elemental composition and surface chemistry. Such characterisation also gives information on the type and amount of impurities and the presence/absence of a coating. The purity range of NM-20x was from 96 to 99 %, see Table 3 for the values for each material. No information concerning purity was given for the materials studied by Korea and Japan.

1.4. Use Pattern

A. Uses

SAS is a High Production Volume (HPV) chemical which is widely used in many industries and in various applications such as synthetic resins, plastics, lacquers, vinyl coatings, varnishes, adhesives, paints, printing inks, silicone rubber, fillers in the rubber industry, tyre compounds, insulation material, additive to coatings, as free-flow and anti-caking agents in powder materials, including food, as tooth paste additives, pharmaceuticals, cosmetics, as liquid carriers particularly in the manufacture of agrochemicals and animal feed, and foods, resulting in widespread exposure to these substances (Fruijtier-Pölloth, 2012). SAS is also increasingly used in diagnostic and biomedical research such as cancer therapy, DNA delivery, and enzyme immobilization (Barik et al., 2008); the total volumes for these uses is though low in comparison with the industrial uses. According to SRI Consulting precipitated SAS is one of the most abundant nanomaterial on the market in terms of quantity[2].

[2] http://www.ihs.com/products/chemical/planning/scup/nanoscale-chemicals.aspx?pu=1&rd=chemihs

The selected SASs in the NM-series are used, among others, in food applications and as reinforcement in car tyres (rubber), e.g. car tyres.

B. Method of production (e.g., precipitation, gas phase):

The methods of production of synthetic amorphous silica are described in detail in e.g. EC, 2007. The principles of particle formation are describy in "The Chemistry of Silica"" by Iler RK, (1979).

Thermal Route

Pyrogenic silica is a very fine particulate form of silicon dioxide and is prepared by burning silicon tetrachloride ($SiCl_4$) or trichlorosilane ($SiHCl_3$) in an oxyhydrogen gas flame:

$$SiCl_4 + 2\ H_2 + O_2 \rightarrow SiO_2 + 4\ HCl$$

$$SIHCl_3 + H_2 + O_2 \rightarrow SiO_2 + 3\ HCl$$

By varying e.g. the flame temperature, flame composition and feed stock, the product's physical-chemical properties, e.g. the specific surface area and the particle size, can be controlled. NM-202 and NM-203 were synthsised via this type of process.

Wet Route

Different manufacturing methods are possible, and (**1**) describes the way the materials NM-200, NM-201 and NM-204 were synthetized; (**2**) this process is used for synthesis of monodisperse particles, with a control of the shape and the size of the nanomaterial; process (**2**) is without commercial relevance.

1. Amorphous silica, silica gel, is produced by the acidification of solutions of sodium silicate to produce a gelatinous precipitate that is then washed with water and afterwards dehydrated to produce colourless microporous silica.

Briefly, the precipitation method reacts an alkali metal silicate dissolved in water, e.g. water glass ($Na_2O \cdot nSiO_2$; n = 2 – 4) with sulphuric acid, through a series of production steps that include raw material storage, synthesis, solid-liquid-filtration, drying and packaging. The synthesis can either be continuous or in batch.

$$Na_2O\ x\ nSiO_2 + H_2SO_4 \rightarrow nSiO_2 + Na_2SO_4 + H_2O$$

2. In order to obtain monodisperse SAS nanoparticles, e.g. sol-gel methods are employed such as the Stöber method, which is not utilized commercially, and the water-in-oil (w/o) micro-emulsion method. The sol-gel process is based on a series of hydrolysis, condensation and polymerisation reactions of an alkoxide. The most widely used precursors are alkoxysilanes, such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS).

Hydrolysis is initiated by the addition of water to the silane solution under acidic, neutral, or basic conditions $Si\text{-}(OR)_4 + H_2O \rightleftharpoons (RO)_3\text{-}Si\text{-}OH + ROH$

During the condensation step a molecule, such as water or alcohol, is liberated. This leads to polymerisation and synthesis of a network of silane (Si-O-Si) and to production of nanomaterials.

$$Si\text{-}(OR)_4 + HO\text{-}Si\text{-}(OR)_3 \rightleftharpoons (RO)_3\text{-}Si\text{-}O\text{-}Si\text{-}(OR)_3 + ROH \quad \text{(with alcohol)}$$

or $$(RO)_3\text{-}Si\text{-}OH + HO\text{-}Si\text{-}(OR)_3 \rightleftharpoons RO)_3\text{-}Si\text{-}O\text{-}Si\text{-}(OR)_3 + H_2O \quad \text{(with water)}$$

2. PHYSICAL CHEMICAL DATA

2.1 Overview of Identification information and Physical Chemical Data for SAS

SAS is well described in the literature with regard to "classical[3]" physical-chemical properties and these are reported also in standard reference works; some of the "classical" physical-chemical properties are not part of the end-points agreed under the Sponsorship Program.

For physical-chemical properties listed in the Sponsorship Program as relevant for nanomaterials, some appear not to be relevant for SAS (crystalline phase and size, photocatalytic activity, redox potential, radical formation). The determination of K_{ow} is not feasible for inorganic nanomaterials, as discussed at an OECD expert meeting in Mexico, March 2013, see OECD 2014, and was considered to be not relevant for insoluble and sparingly soluble nanomaterials. For the relevant parameters, a wide range of values are presented in the background reports (OECD (2004a), ECETOC (2006), SASSI (2008)) obtained through measurements of different sources of SAS, see for example ECETOC (2006) p. 12. These reports present only limited physical-chemical characterisation data associated to each of the different specific SASs that were tested; thus this data is not fulfilling the information requests in the Sponsorship Program, and the information available from the reports would not necessarily relate to the principal material.

In the Joint Action NANOGENOTOX precipitated and pyrogenic synthetic amorphous silicon dioxide were characterised, see Rasmussen, 2013. The Joint Action developed a dispersion protocol, found at http://www.nanogenotox.eu/files/PDF/web%20nanogenotox%20dispersion%20protocol.pdf . For the characterisation of the dispersed materials, it cannot be excluded that the dispersion procedure influences the material properties.

This section describes the characterisation results of the principal material (NM-200) and alternate materials (NM-201, NM-202, NM-203 and NM-204), and presentes the data provided for characterising the Korean material.

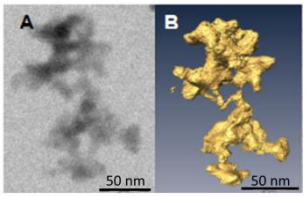
The following Table 4 gives an overview of the information available for the end-point group *Nanomaterial Information / Identification*. Table 3 summarises the *physical chemical characterisation* data generated for the principal material (NM-200) in the Testing Programme.

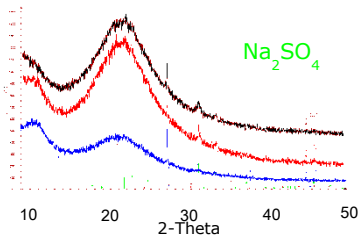
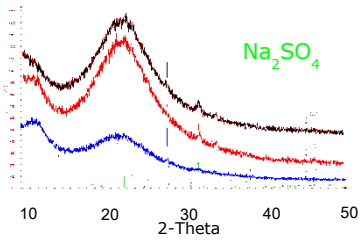
The Table 5 summarises the *physical chemical characterisation* data generated for the WPMN Testing Programme for the principal and alternate materials. An overview of the test methods used for obtaining experimental data is given in Table 1. The data for NM-201, NM-202, NM-203 and NM-204, presented in the same way as data for NM-200 in Table 3, are given in Appendix 0.

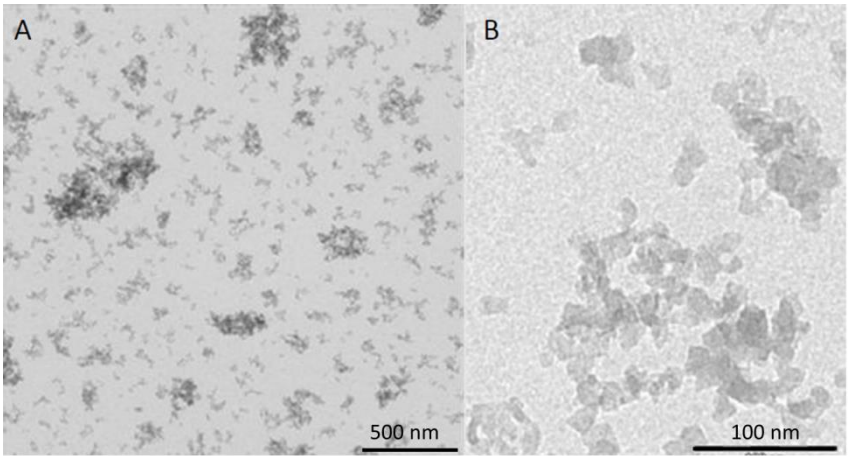
For DLS measurements, the instrument measures the Z-average (diameter of particles scattering with higher intensity) over three runs, and this value is stated together with the minimum (-) and maximum (+) values of the Z-average of the three runs. The polydispersity index (PdI) is a measure of the width of the particle size distribution, and is based on assumptions of a model: A monomodal model, called the cumulant analysis, is often used to treat the raw data correlograms (decaying as exponential). It determines a Z-average and a polydispersity index. For polydisperse samples, more sophisticated, multimodal analysis models, e.g the CONTIN method, can be applied to reveal size distributions. Polydispersity indices less than 0.1 are typically referred to as "monodisperse". Details are published in Annex 5 to the dossier (OECD WPMN, 2015).

[3] Melting point, boiling point, vapour pressure, flash point, auto flammability, flammability, explosive properties, oxidising properties, viscosity, water solubility, octanol-water partition coefficient.

Table 3. Overview of results of physical-chemical characterisation of the principal material, NM-200; dispersion, when relevant, was mostly done following the Nanogenotox dispersion protocol. Testing by the JRC may not have used this dispersion protocol. BIAC did not use the Nanogenotox dispersion protocol.

Method	Institution	Results
Homogeneity		
DLS	CEA, INRS, NRCWE	Repeated DLS studies were performed between the different vials and from different areas within the vial. The observed variability between the different vials is very low (2-3%) but intra-vial is much higher: 6-10%.
Agglomeration / aggregation		
SAXS	CEA	Structure and size parameters extracted from SAXS data. Gyration radius of primary particles and aggregates $2xRg_1$: 18 nm and $2xRg_2$: 440 nm, fractal dimension D_f: 2.45 and number $N_{part/agg}$ of particles per aggregate: 3600
DLS	CEA	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 207.1 ± 12.3, PdI: 0.390 ± 0.041
	NRCWE	• Ultra-pure water dispersion (inter vial study) Z-average (nm): 181.5 ± 4.3, PdI: 0.238 ± 0.006
	INRS	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 240.5 ± 2.3, PdI: 0.248 ± 0.006
	JRC	• Milli-Q water dispersion. Z-average (nm): peak 1: 136, peak 2: 376, PdI: 0.524 • culture media dispersion Z-average (nm): peak 1: 144.4, peak 2: 2611, PdI: 0.492 • PBS dispersion Z-average (nm): peak 1: 187.2, peak 2: 712.7, PdI: 0.532
	BIAC, Rhodia operations	Method: Dynamic light scattering (DLS), Concentration: 0.3wt%, Liquid phase: Water, Dispersion: Ultrasonic (Vibracell), 240 sec, energy input:600W. Mean diameter reported: 15.22 micrometers.
TEM	CODA-CERVA IMC-BAS	High porosity nanostructured material which may be considered aggregates of primary particles. Mean diamater (nm): 31 ± 3. Feret min: 21.9 nm (median of 8005) Feret max: 34.5 nm (median of 8005) Morphology of aggregates/agglomerates: low to medium sphericity, sub-angular to rounded.
	BIAC, Rhodia operations	Agglomerated Silica; the elementary particle size varies from 5 to 20 nm with an average size mainly around 10 -15 nm.
TEM-tomography	CODA-CERVA	 Aggregates of very complex morphology composed of a variable number of interconnected primary subunits.

AFM	CEA	Third dimension of the agglomerates/aggregates: median (of 1382): 21.9 nm
Laser diffraction spectrometry	BIAC, TU Dresden, Germnay	The test method is in accordance with EN 481, ISO 9276-2, based on the principle of light scattering measured on the dry powder. The median particle diameter weighted by volume amounts to 480 micrometers. (The instrument used, LAP 321, has a lower size limit of 0.3 micrometers, according to the instrument manufacturer (http://www.exisab.com/Docs/Newsletters/ExIS_News_March_2011.pdf).)
Water Solubility		
Motomizu	BIAC, Rhodia Operations	The saturation concentrations $[M]_{tot}$ for test substance equivalent to NM-200 has been determined to 2.4 mmol/l. (The method is a Spectrophoto-metric Determination of Silicate in water with Molybdate and Malachite Green)
24-hour acellular *in vitro* incubation test in special solutions	NRCWE	The 24-hour dissolution ratio of NM-200 was measured in three different media: 0.05% BSA in water, Gambles solution and Caco 2 media. Both NM-200 and the Al impurities are partially soluble in all media but amounts vary considerably with medium, as does the relative amounts of dissolved Al impurities compared with dissolved Si, suggesting that the solubility behaviour of the Al impurity and NM-200 depends on the medium.
Crystalline phase		
XRD	JRC	Synthetic amorphous silicon dioxide. Peaks supporting the presence of crystalline material, consistent with Na_2SO_4 were seen.
	NRCWE	 Synthetic amorphous silicon dioxide; impurities of Na_2SO_4.
	IMC-BAS	Synthetic amorphous silicon dioxide.
	BIAC, Rhodia Operations	NM-200 is fully amorphous; no crystalline structure can be determined
Dustiness		
Small Rotating Drum	NRCWE	Inhalable dustiness index (n=3) 6459 ± 273 (mg/kg) Respirable dustiness index (n=3) 293 ± 193 (mg/kg)
Vortex Shaker Method	INRS	Respirable dustiness index (n=1) 34000 ± 0.0304 (mg/kg)
Crystallite size		
SAXS	CEA	Amorphous material. Primary particle size: Equivalent diameter for spheres: 22nm, $2xRg_1$ is 18 nm
XRD	JRC	Synthetic amorphous silicon dioxide. Traces of crystalline material seen around 2-Theta equal to 32° and 34°, which is consistent with the suggested presence of Na_2SO_4
	NRCWE	 Synthetic amorphous silicon dioxide. Crystalline impurities of Na_2SO_4

	IMC-BAS	Synthetic amorphous silicon dioxide
Representative TEM picture(s)		
TEM	CODA-CERVA, IMC-BAS	 Aggregates with dense, complex structure
Particle size distribution		
SAXS	CEA	Primary particle size: Equivalent diameter for spheres: 22 nm, 2xRg_1 is 18nm
TEM	CODA-CERVA	Primary particle size: 14 ± 7 nm
	IMC-BAS	Primary particle size: 18 nm
	INRS	Primary particle size: 23 ± 8 nm
TEM	CODA-CERVA IMC-BAS	Number (expressed in %) of SAS NM particles smaller than 100 nm, 50 nm and 10 nm <100 nm - 88.7%, <50 nm - 69.8% <10 nm - 1.7%
DLS	CEA	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and reveals the presence of large aggregates of few microns.
	JRC	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. (see Aggregation/ Agglomeration results)
	NRCWE	The material is polydisperse. (see Aggregation/ Agglomeration results)
	INRS	The material is polydisperse. (see Aggregation/ Agglomeration results)
CLS	JRC	Peak (nm): 75 - 95, CLS Pdl: 10.18
Specific Surface Area		
BET	IMC-BAS	189.16 (m^2/g)
SAXS	CEA	123.3 ± 4.9 (m^2/g)
Volume Specific Surface Area		
TEM tomography	CODA-CERVA	342 ± 36 (m^2/cm^3) (Volume specific surface area)
Zeta Potential (surface charge)		

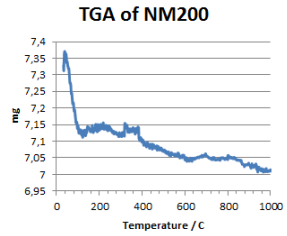
Zetametry	CEA	NM-200 forms a stable suspension, with negatively to neutral charged nanoparticles. The zeta potential, however, varied greatly as function of pH and reached - 45 mV around pH 7. IEP <2
	JRC	Zeta potential at pH 7, milliQ water: -47.5 (mV). Zeta potential at pH 7.1, PBS: - 18 (mV)
Surface Chemistry		
XPS	JRC	The following elements were identified in the surface of NM-200: O (70.8 at %), Si (24.1 at %), C (4.1 at %), Na (1.0 at %) and S (0.1 at %). The presence of C, carbon, is considered to be due to surface contamination from hydrocarbons from air that have attached themselves to the material surface.
TGA	NRCWE	 Significant mass loss was observed below 100°C (water). A 2 wt % gradual mass loss was observed above 110°C and may indicate e.g. loss of water associated in the micro pores or is associated with the presence of Na_2SO_4.
Photo-catalytic activity		
End-point not relevant for SAS		
Pour-density		
Weighing	INRS	0.12 g/cm^3 (8 wt% water content)
Porosity		
BET	IMC-BAS	Micropore volume (mL/g): 0.01181
Octanol-water partition coefficient,		
End-point not relevant		
Redox potential		
OxoDish fluorescent sensor plate for O_2 detection	NRCWE	The evolution of O_2 level during 24-hour incubation was measured in three different media. Different dO_2 values were observed for these media. In the 0.05% BSA-water and Gambles solution NM-200 showed negligible reactivity. In Caco 2 media, a negative dose-response relation was observed with decreasing dO_2 level with increasing concentration of NM-200. The results suggest that NM-200 is inactive or reductive in the different incubation media.
Radical formation		
HPLC + UV	NRCWE	Using the benzoic acid probe to form 4 hydroxy benzoic acid in a phosphate buffered hydrous solution gave no detectable concentration OH radicals.
Composition		
EDS	IMC-BAS	Na - 8800 ppm, Al - 4600 ppm, S - 8700 ppm, Si - 44.77 (wt %), O (wt%) calculated - 53.02
ICP-OES		Impurities > 0.01 %: Al, Ca, Na (> 0.1 %), S (> 0.1 %) Impurities 0.005 - 0.01 % : Fe, K Impurities 0.001 – 0.005 %: Mg, Zr

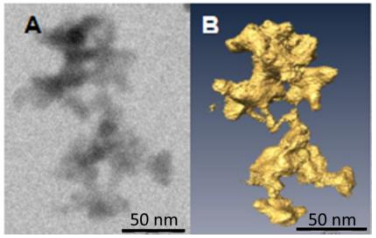
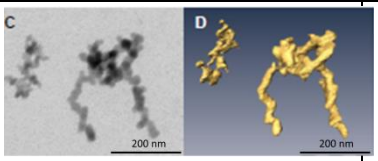
Table 4. Summary of the Nanomaterial Information / Identification end-points for the Synthetic Amorphous Silica (SAS) investigated.

NANOMATERIAL / **ENDPOINTS**	**SiO_2 Principal Material**	**SAS, SiO_2 Alternate Materials**			
	NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
Nanomaterial name	Silicon Dioxide, Synthetic Amorphous Silica (SAS), NM-200	Silicon Dioxide, Synthetic Amorphous Silica (SAS), NM-201	Silicon Dioxide, Synthetic Amorphous Silica (SAS), NM-202	Silicon Dioxide, Synthetic Amorphous Silica (SAS), NM-203	Silicon Dioxide, Synthetic Amorphous Silica (SAS), NM-204
CAS Number	General CAS No. for SiO_2: 7631-86-9				
CAS Number	112926-00-8 for precipitated silicon dioxide	112926-00-8 for precipitated silicon dioxide	112945-52-5 for pyrogenic silicon dioxide	112945-52-5 for pyrogenic silicon dioxide	112926-00-8 for precipitated silicon dioxide
Structural formula / molecular structure	SiO_2, strong, directional covalent bonds, and has a well-defined local structure: four oxygen atoms are arrayed at the corners of a tetrahedron around a central silicon atom				
Composition	Purity: $\geq$ 96% SiO_2, 2.7% Na_2SO_4 0.87 % Al[4]	Purity: $\geq$ 97% SiO_2, 1.4% Na_2SO_4 0.74 % Al[4]	Purity: $\geq$ 99% SiO_2 0.45 % Al[4]	Purity: $\geq$ 99% SiO_2 0.43% Al[4]	Purity: $\geq$ 98% SiO_2 0.6% Na_2SO_4 0.48% Al[4]
Analytical Method(s) of detection	Overview in NIOSH manual of analytical methods http://www.cdc.gov/niosh/docs/2003-154/pdfs/7501.pdf				
Basic morphology	White, fluffy, amorphous powder				
Surface chemistry	Neither coated nor modified.	Neither coated nor modified.	Neither coated nor modified.	Neither coated nor modified.	Results from GC-MS analysis were inconclusive.
Commercial uses	Multiple. It is a High Production Volume (HPV) chemical; for example car tyres (rubber), printing inks, paints.				
	Cosmetics, food, animal feed, etc.	Rubber	Food, rubber	Cosmetics, Animal feed, food,	Animal feed, tyres
Known catalytic activity	None	None	None	None	None
Production method	Precipitation	Precipitation	Flame hydro-lysis / thermal process	Flame hydro-lysis / thermal process	Precipitation

[4] The presence of aluminia is confirmed in a study by C. Motzkus et al. of the NM-20x series "Impact of batch variability on physicochemical properties of manufactured TiO_2 and SiO_2 nanopowders" published in Powder Technology 267 (2014) 39–53, analysed the chemical composition by X-ray fluorescence and found that Al_2O_3 was among the impurities present in a concentration higher than 0.05% for all five NM-20x.

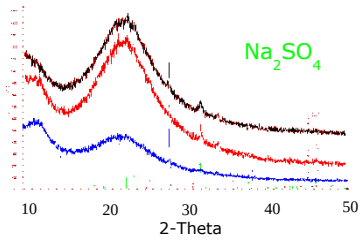
Table 5. Summary of the Physical-chemical Properties and Material Characterization Endpoints for the SAS-NMs from the JRC Repository generated in the Nanogenotoc project. Dispersion, when relevant, was mostly done following the Nanogenotox dispersion protocol. Testing by the JRC may not have used this dispersion protocol. BIAC did not use the Nanogenotox dispersion protocol.

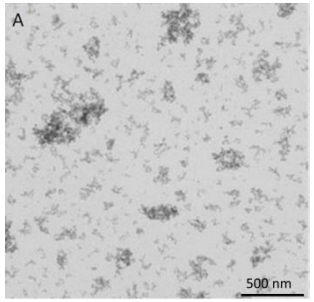
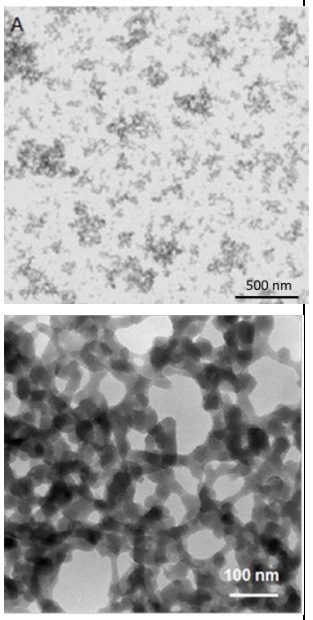
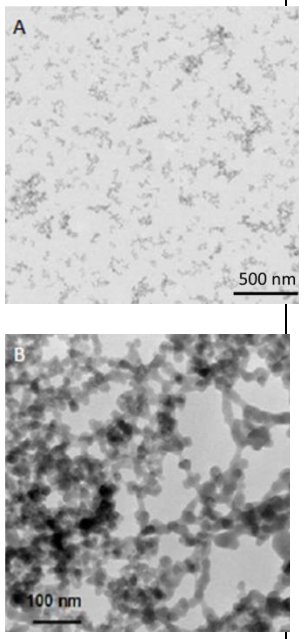
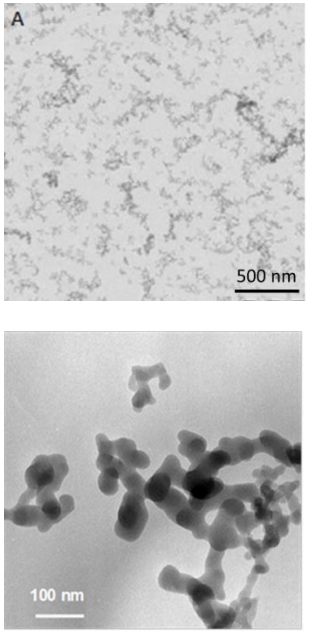
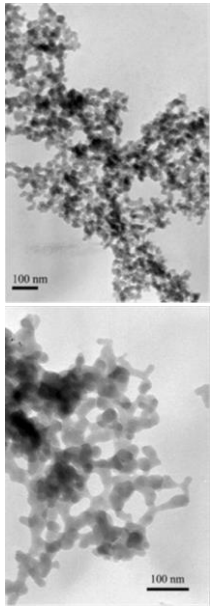
NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
PHYSICAL-CHEMICAL PROPERTIES					
1. Agglomeration/ Aggregation (DLS)	Results from 3 institutions using Ultra-pure water dispersion: Z-average (nm): 207.1 ± 11.9, PdI: 0.390±0.041(intra vial study) Z-average (nm): 181.5 ± 4.3, PdI: 0.238 ± 0.0.006 (inter vial study) Z-average (nm): 240.5 ± 2.3, PdI: 0.248±0.0.006(intra vial study) BIAC reports that the diameter measured by DLS is 15.22 micrometers	Results from 1 institution and ultra-pure water dispersion: Z-average (nm): 208.1 ± 34.5, PdI: 0.352 ± 0.028 (intra vial study) Z-average (nm): 197.0 ± 15.7, PdI: 0.337 ± 0.020 (inter vial study)	Results from 1 institution and ultra-pure water dispersion: Z-average (nm): 175.9 ± 4.5, PdI: 0.355 ± 0.001 (intra vial study)	Results from 3 institutions and Ultra-pure water dispersion: Z-average (nm): 172.9 ± 9.2. PdI: 0.427 ± 0.025 (intra vial study) Z-average (nm): 147.5 ± 4.5. PdI: 0.244 ± 0.017 (intra vial study) Z-average (nm): 146.8 ± 0.6, PdI: 0.229 ± 0.015 (inter vial study) Z-average (nm): 245.7 ± 37.2. PdI: 0.299 ± 0.024 (intra vial study)	-
(SAXS)	Measurement: Structure and size parameters from SAXS data.				
	Gyration radius of primary particles and aggregates $2xRg_1$: 18 nm and $2xRg_2$: 440 nm, fractal dimension D_f: 2.45 and number $N_{part/agg}$ of particles per aggregate: 3600	Gyration radius of primary particles and aggregates $2xRg_1$: 20 nm and $2xRg_2$: 180 nm, fractal dimension D_f: 2.45 and number $N_{part/agg}$ of particles per aggregate: 457	Gyration radius of primary particles and aggregates Rg_1: 16 nm and Rg_2: 100 nm, fractal dimension D_f: 2.5 and number $N_{part/agg}$ of particles per aggregate: 200	Gyration radius of primary particles and aggregates Rg_1: and Rg_2: fractal dimension D_f and number $N_{part/agg}$ of particles per aggregate could not be calculated as parameters could not be fitted.	-
(TEM)	Sub-rounded shape with a low to medium sphericity (de Temmermann, 2012).	Rounded to well rounded shaped with a medium sphericity (de Temmermann, 2012).	Very angular to subangular shape with a low sphericity and complex and branched structure (de	Very angular to subangular shape with a low sphericity and complex and branched structure (de Temmermann, 2012).	The number of particles smaller than 100 nm is 71.2 %. Manual measurement of the

NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
	Median mean diamater (nm): 31 ± 3[5]. Feret min: 21.9 nm (median of 8005) Feret max: 34.5 nm (median of 8005) Morphology of aggregates/agglomerates: Low to medium sphericity, sub-angular to rounded. % of aggegates < 100 nm: 88.7 % BIAC data: Agglomerated Silica; the elementary particle size varies from 5 to 20 nm with an average size mainly around 10-15 nm.	Median diamater (nm): 43 ± 4. Feret min: 25.4 nm (median of 5311) Feret max: 38.5 nm (median of 5311) Morphology of aggregates/agglomerates: Medium sphericity, rounded to well--rounded. % of aggegates < 100 nm: 81.5 %	Temmermann, 2012) Median diamater (nm): 53 ± 9. Feret min: 37.2 nm (median of 4248) Feret max: 58.4 nm (median of 4248) Morphology of aggregates/agglomerates: Low sphericity—very angular to sub-angular. % of aggregates <100 nm: 80.4 %	Median diamater (nm): 48 ± 4 Feret min: 33.5 nm (median of 4889) Feret max: 53.2 nm (median of 4889). % of aggregates < 100nm: 88 ± 2. Morphology of aggregates/agglomerates: Low sphericity, angular. % of aggregates <100 nm: 77.5 %	ESD gives a result in the range of 10 nm – 15 nm % of aggregates <100 nm: 71.2 %
(TEM-tomography)		-	-	 Aggregates of very complex morphology composed of a variable number of interconnected	-

[5] Characterization of aggregated SAS NM by quantitative TEM. Mean values of medians ± SD

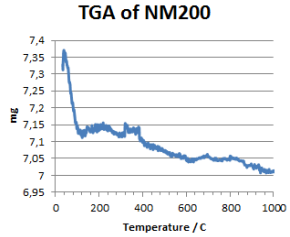
NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
	Aggregates of very complex morphology composed of a variable number of interconnected primary subunits.			primary subunits.	
AFM	median (of 1382): 21.9 nm	median (of 1275): 33.5 nm	median (of 1103): 38.2 nm	median (of 593): 24.2 nm.	
2. Water Solubility/ Dispersability (test performed: 24-hour acellular *in vitro* incubation test in special solutions)	Measurement: The 24-hour dissolution ratio was measured in three different media: 0.05% BSA in water, Gambles solution and Caco 2 media.				
	Both NM-200 and the Al impurities are partially soluble in all media but amounts vary considerably with medium, as does the relative amounts of dissolved Al impurities compared with dissolved Si, suggesting that the solubility behaviour of the Al impurity and NM-200 depends on the medium.	Both NM-201 and the Al impurities are partially soluble in Gambles Solution and Caco2 media but amounts vary considerably with the medium. In 0.05% BSA in water only the Al impurities are partially soluble; Si was below the detection limit. The relative amounts of dissolved Al impurities and dissolved Si are different depending on medium, which suggests different solubility behaviour of Al impurities and NM-201 depending on the medium.	Both NM-202 and the Al impurities are partially soluble in all media but amounts vary considerably with medium as does the relative amounts of dissolved Al impurities and dissolved Si suggesting different solubility behaviour of Al impurities and NM-202 depending on the medium.	Both NM-203 and the Al impurities are partially soluble in all media but amounts vary considerably with medium, as does the relative amounts of dissolved Al impurities compared with dissolved Si, suggesting that the solubility behaviour of the Al impurities and NM-203 depend on the medium.	Both NM-204 and the Al impurities are partially soluble in 0.05% BSA in water and Caco2 media but amounts vary considerably with medium. In Gambles solution only NM-204 is partially soluble. The relative amounts of dissolved Al impurities and dissolved Si differ depending on medium, which suggests different solubility behaviour of Al impurities and NM-204 depending on the medium.

NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
3. Crystalline phase (XRD)	 Measurements by 3 institutes: The SiO_2 phase is amorphous. Impurities: peaks consistent with the presence of Na_2SO_4 were observed.	Measurements by 3 institutes: The SiO_2 phase is amorphous. Impurities: peaks consistent with the presence of Na_2SO_4 were observed.	Measurements by 3 institutes: The SiO_2 phase is amorphous.	Measurements by 3 institutes: The SiO_2 phase is amorphous.	Measurements by 3 institutes: The SiO_2 phase is amorphous.
4. Dustiness (Small Rotating drum, arbitrary units)	Inhalable dustiness index (n=3) 6459 ± 273 Respirable dustiness index (n=3) 293 ± 193	Inhalable dustiness index (n=3) 6034 ± 199 Respirable dustiness index (n=3) 218 ± 24	Inhalable dustiness index (n=3) 4988 ± 1866 Respirable dustiness index (n=3) 91 ± 11	Inhalable dustiness index (n=3) 5800 ± 1488 Respirable dustiness index (n=3) 354 ± 6	Inhalable dustiness index (n=3) 24969 ± 601 Respirable dustiness index (n=3) 1058±-
(VORTEX shaker method, arbitrary units)	Respirable dustiness index (n=1) 34000	Respirable dustiness index (n=1) 6500	Respirable dustiness index (n=1)17000	Respirable dustiness index (n=1) 51000	Respirable dustiness index (n=1) 14000
5. Crystallite size (XRD)	Measurements by 3 institutes: The SiO_2 phase is amorphous.	Measurements by 3 institutes: The SiO_2 phase is amorphous.	Measurements by 3 institutes: The SiO_2 phase is amorphous.	Measurements by 3 institutes: The SiO_2 phase is amorphous.	Measurements by 3 institutes: The SiO_2 phase is amorphous.

NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
6. Electron Microscopy (TEM) micrographs (TEM))	 Aggregates with complex structure	 Aggregates with complex network structure.	 Aggregates with a complex and branched structure	 Aggregates with a complex and branched structure.	 Aggregates with complex, open structure.
7. Particle size distribution (SAXS)	Primary particle size: Equivalent diameter for spheres: 22 nm Gyration radius for primary particles $2xRg_1$ = 18 nm	Primary particle size: Equivalent diameter for spheres: 22 nm Gyration radius for primary particles $2xRg_1$ = 20 nm	Primary particle size: Equivalent diameter for spheres: 15 nm Gyration radius $2xRg_1$ = 16 nm	Primary particle size: Equivalent diameter for spheres: 16 nm Parameters could not be fitted	Primary particle size: Equivalent diameter for spheres: 21 nm

NANOMATERIAL / **ENDPOINTS** (method)	**SiO_2 Principal Material**	**SiO_2 (Silicon Dioxide (SAS)) Alternate Materials**			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
(TEM)	Primary particle size measured by three institutions: 14 ± 7 nm, 23 ± 8 nm, 18 nm	Primary particle size measured by three institutions: 17 ± 8 nm, 19 ± 4 nm, 18 nm	Primary particle size measured by three institutions: 15 ± 7 nm, 18 ± 3 nm, 20 nm	Primary particle size measured by three institutions: 13 ± 6 nm, 16 ± 3 nm, 45 nm	Primary particle size 19 nm and by manual measurements 10 -15 nm.
(DLS)	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. The material is dispersed in ultra-pure water. The results from 3 institutes are: Z-average (nm): 207.1 ± 12.3, Pdl: 0.390 ± 0.041, FWHM peak width (nm): 159.8 ± 50.11 Z-average (nm): 181.5 ± 4.3, Pdl: 0.238 ± 0.006, main peak (nm): 116.7 ± 8.3 Z-average (nm): 240.5 ± 2.3, Pdl: 0.248 ± 0.006	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. The material is dispersed in ultra-pure water. The results from 1 institute are: Z-average (nm): 197.0 ± 15.7, PdI: 0.337 ± 0.020 FWHM peak width (nm): 105.6 ± 49.3	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. The material is dispersed in ultra-pure water. The result from 1 institute is: Z-average (nm): 175.9 ± 4.5, PdI: 0.355 ± 0.001, FWHM peak width (nm): 56.2 ± 2.9	The material is polydisperse. The intensity size distribution, consisting of two main peaks is very broad and reveals the presence of large aggregates of few microns. The material is dispersed in ultra-pure water. The results from 3 institutes are: Z-average (nm): 245.7 ± 37.2. PdI: 0.299 ± 0.024 Z-average (nm): 147.5 ± 4.5. PdI: 0.244 ± 0.017, FWHM: 84.4 ± 10.4 Z-average (nm): 146.8 ± 0.6 PdI: 0.229 ± 0.015, FWHM: 83.8 ± 0.6	
8. Specific surface area (BET)	189.16 m^2/g	140.46 m^2/g	204.11 m^2/g	203.92 m^2/g	136.6 m^2/g
(SAXS)	123.3 ± 4.9 m^2/g	123.3 ± 8.3 m^2/g	184 ± 17.8 m^2/g	167.2 ± 13.4 m^2/g	131 ± 22.9 m^2/g

NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
(TEM-tomography)	VSSA: 342 ± 36 m^2/cm^3	-	-	VSSA: 219 ± 23 m^2/cm^3	-
9. Zeta-Potential (Surface charge) (Zeta-potential/electrical mobility)	When dispersed according to the NANOGENOTOX dispersion protocol, NM-200 forms a stable suspension (no sedimentation, the values of the Z-average and mean count rate were unchanged over 16 h), with negatively to neutral charged nanoparticles. The zeta potential varied greatly as function of pH and reached -45 mV around pH 7. IEP <2	When dispersed according to the NANOGENOTOX dispersion protocol, NM-201 forms a stable suspension (no sedimentation, the values of the Z-average and mean count rate were unchanged over 16 h), with negatively to neutral charged particles. The zeta potential varied greatly as function of pH and reached -40 mV around pH 7. IEP <2	When dispersed according to the NANOGENOTOX dispersion protocol, NM-202 forms a stable suspension (no sedimentation, the values of the Z-average and mean count rate were unchanged over 16 h), with negatively to neutral charged particles. The zeta potential varied greatly as function of pH and reached -40 mV around pH 7. IEP <2	When dispersed according to the NANOGENOTOX dispersion protocol, NM-203 forms a stable suspension (no sedimentation, the values of the Z-average and mean count rate were unchanged over 16 h), with negatively to neutral charged particles. The zeta potential varied greatly as function of pH and reached -35 mV around pH 7. IEP 2-4	-
10. Surface chemistry (XPS)	The following elements were identified in the surface: O (70.8 at%), Si (24.1at%), C (4.1 at%), and Na (1 at%). The presence of C is considered to be due to surface contamination from ambient air.	The following elements were identified in the surface of NM-201: O (70.3 at%), Si (23.6 at%), C (4.5 at%) and Na (1.5 at%). The presence of C is considered to be due to surface contamination from ambient air.	The following elements were identified in the surface of NM-202: O (72.1 at%), Si (25 at%),and C (2.9 at%). The presence of C is considered to be due to surface contamination from ambient air.	The following elements were identified in the surface of NM-203: O (71.7 at%), Si (26 at%) and C (2.3 at%). The presence of C is considered to be due to surface contamination from ambient air.	The following elements were identified in the surface of NM-204: O (71.9 at%), Si (23.2 at%), Na (0.5 at%) and C (4.3 at%). Presence of C is considered to be due to surface contamination from ambient air.

NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
(TGA)	 4% mass loss below 100°C (water). Gradual mass loss above 110°C indicating e.g. loss of water associated in the micro pores or associated with the presence of Na_2SO_4.	2% mass loss below 100°C (water). Gradual mass loss above 110°C indicating e.g. loss of water associated in the micro pores or associated with the presence of Na_2SO_4.	No mass loss observed	No mass loss detected. Phase transtion detected at 324°C (DTA)	2% mass loss below 100°C (water). Gradual mass loss above 110°C, more than 1%. indicating e.g. loss of water associated in the micro pores or associated with the presence of Na_2SO_4
11. Photocatalytic activity	n/a	n/a	n/a	n/a	n/a
12. Pour density (Weighing)	0.12 g/cm^3 (8 wt% water content)	0.28 g/cm^3 (8 wt% water content)	0.13 g/cm^3 (1 wt% water content)	0.03 g/cm^3 (1 wt% water content)	0.16 g/cm^3 (6 wt% water content)
13. Porosity (BET)	Micropore volume (mL/g): 0.01181	Micropore volume (mL/g): 0.00916	Micropore volume (mL/g): 0.00084	Micropore volume (mL/g): 0.0	Micropore volume (mL/g): 0.00666
14. n-octanol-water partition coefficient	n/a	n/a	n/a	n/a	
15. Redox potential (OxoDish fluorescent sensor plate for O_2 detection)	The evolution of O_2 level during 24-hour incubation was measured in three different media. Different dO_2 values were observed for all applied media.				
	In Gambles solution the concentration of dO_2 increases together with concentration of NM-200. Conversely, for Caco 2 media dO_2 level decreases while the concentration of NM-200 increases. For 0.05% BSA water media almost no changes in the dO_2 levels	In Gambles solution and Caco 2 media the concentration of dO_2 peaked for 0.16mg/ml concentration of NM-201. In the 0.05% BSA in water the dO_2 level increases along with the	In Caco 2 media the concentration of dO_2 peaked for 0.16 mg/ml concentration of NM-202. In Gambles solution and in 0.05% BSA in water the dO_2 level increased	In all three media the level of dO_2 increased along with the concentration of NM-203. The maximum O_2 changes observed for NM-203 are in the order of 40 µmol/ml, which	A slight reduction of dO_2 was observed for the 3 concentrations of NM-204 in 0.05% BSA in water and Gambles solution. For Caco2 media an increase in dO_2 level was observed only

NANOMATERIAL / ENDPOINTS (method)	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
	were observed. The maximum O_2 changes observed for NM-200 are in the order of 40 µmol/ml, which suggests that the particle reactivity can exceed 1µmol/mg.	concentration of NM-201. The maximum O_2 changes observed for NM-are in the order of 40 µmol/ml, which suggests that the particle reactivity can exceed 1µmol/mg.	along with the concentration of NM-202. The maximum O_2 changes observed for NM-202 are in the order of 40 µmol/ml, which suggests that the particle reactivity can exceed 1µmol/mg.	suggests that the particle reactivity can exceed 1µmol/mg.	for the lowest concentration. The maximum O_2 changes observed for NM-204 are in the order of 40 µmol/ml, which suggests that the particle reactivity can exceed 1µmol/mg.
16. Radical formation potential	n/a	n/a	n/a	n/a	n/a
17. Other relevant information	-	-	-	-	-

2.2. Characterisation data for the material from Korea

Manufacturer: Synthesised in the test laboratory in KRISS (as alternate materials). The protocols and procedures are given in Appendix 0.

Information on Particle size distribution – dry and in relevant media

Results

a) TEM size measurement results (size and distribution of particle size)

TEM images of SiO_2 nanoparticles on carbon-coated copper grids are shown in Figure 5. The nominal sizes of the synthetic SiO_2 nanoparticles were 30 and 40 nm, respectively, and the particles were spherical. The average SiO_2 nanoparticle sizes were 28.6 ± 0.67 nm and 39.4 ± 0.77 nm based on the analysis of 85 and 103 particles in TEM images for the 30 and 40 nm nanoparticles, respectively.

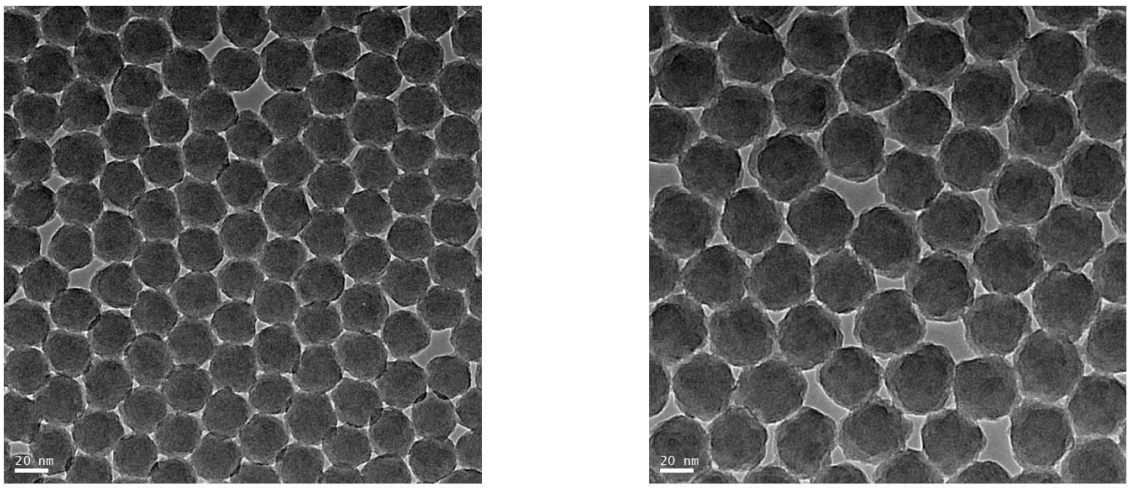


Figure 5. TEM images of 30 nm (left) and 40 nm (right) SiO_2 particles on carbon-coated copper TEM grids

As shown in Figure 6 the size distribution histogram fitted well with a normal distribution (R^2 = 0.8719). This result gave the standard deviation of the primary particle size as 3.1 ± 0.47 nm and 4.0 ± 0.55 nm for the 30 and 40 nm nanoparticles, respectively, meaning that 95 % of the particles were between 20.9 and 36.3 nm for 30 nm SiO_2 and between 29.7 and 49.1 nm for 40 nm SiO_2.

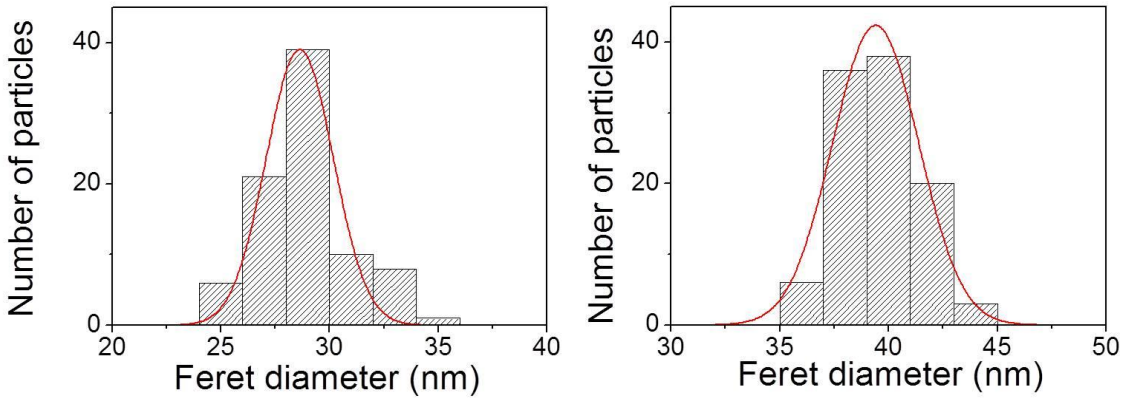


Figure 6. The particle size distribution estimated with the Feret diameters in the images for 30 nm (left) and 40 nm SiO_2 (right) nanoparticles. The Feret diameter is the longest distance between any two points in a particle image boundary.

The average particle size and size distribution was estimated with the Feret diameter, taken from particles identified on TEM images, as a function of storage time for both 30 nm and 40 nm SiO_2 nanoparticles, see Figure 7.

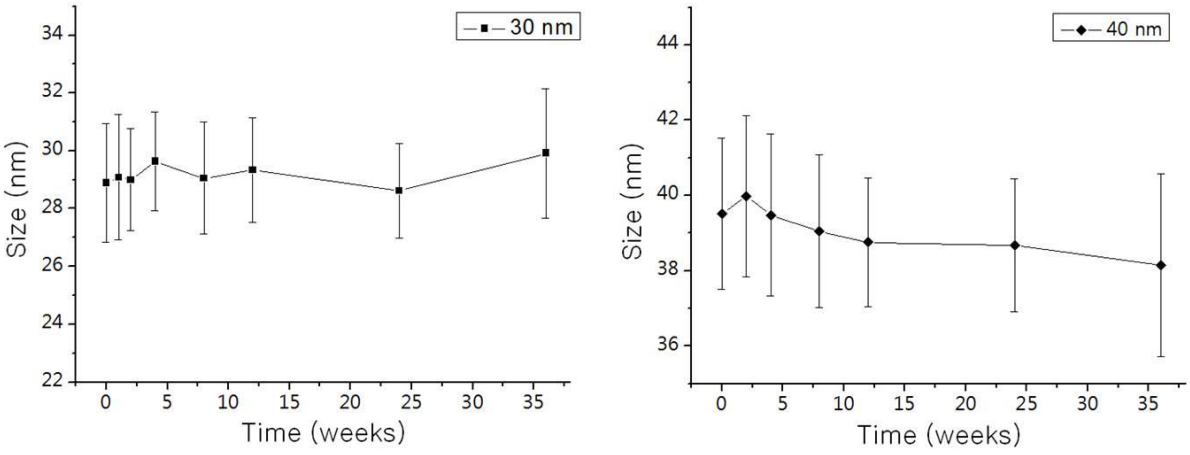


Figure 7. The average particle size and size distribution estimated with the Feret diameter in TEM images as a function of storage time for (left) 30 nm and (right) 40 nm SiO_2 nanoparticles.

b) The results of DLS size measurement

The DLS size of nominal 30 nm SiO_2 was 30.1 ± 0.7 (right-angle detection) and 37.2 ± 3.8 nm (backscatter mode detection). The DLS size of nominal 40 nm SiO_2 was 39.4 ± 1.2 (right angle detection) and 52.4 ± 0.5 nm (backscatter mode detection). The DLS size differed by up to 33 % depending on the detection angles. The suspension stability was investigated for 1 year by observing the UV-Vis absorption spectra and DLS size (Figure 8 and Figure 9). For the 30 nm SiO_2 nanoparticles in aqueous suspension, the UV-Vis absorption spectra were almost invariant, indicating that the SiO_2 nanoparticles rarely underwent sedimentation. In addition, the DLS size of the SiO_2 nanoparticles was constant within 15 % of the average value for 1 year, see Figure 8. In contrast, the UV-Vis absorption spectra of 40 nm SiO_2 nanoparticles in aqueous suspension decreased slightly with storage time, showing that SiO_2 nanoparticles underwent slight sedimentation. The DLS size of 40 nm SiO_2 nanoparticles was constant within 33 % of the average value for 9 months (Figure 9).

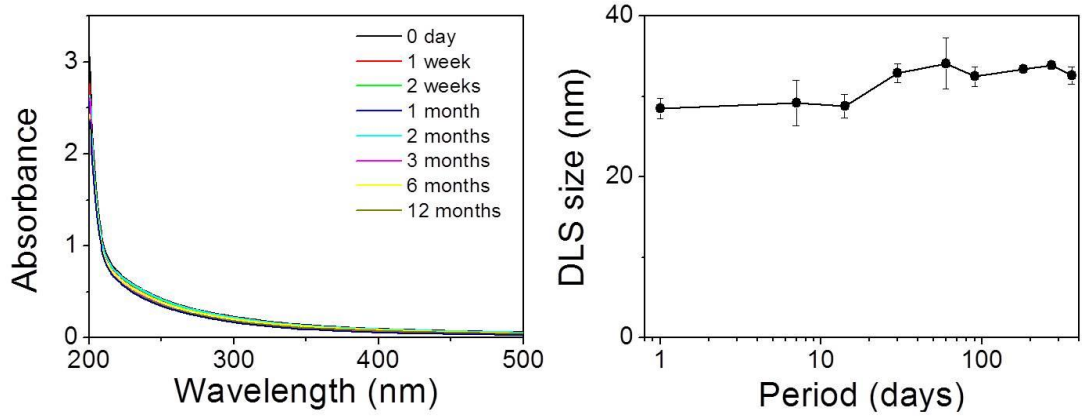


Figure 8. Time dependence of UV-Vis absorption spectra (left) and DLS size of 30 nm SiO_2 in aqueous suspension (right).

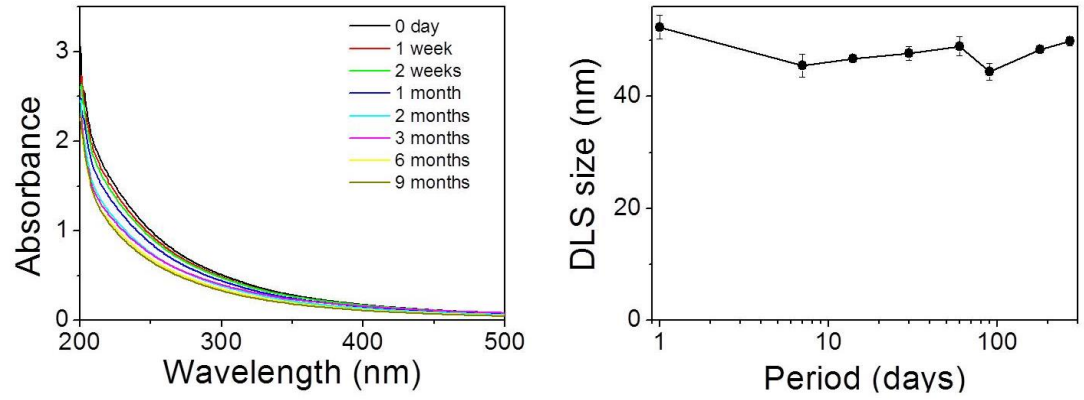


Figure 9. Time dependence of UV-Vis absorption spectra (left) and DLS size of 40 nm SiO_2 in aqueous suspension (right).

Material description based on test results

The alternate materials, SiO_2 nanoparticles synthesised at KRISS, have been characterised as spherical and have nominal diameters of 30 and 40 nm. The size and size distribution showed that the normalised standard deviation ($\sigma(d)/\langle d\rangle$, where $\sigma(d)$ is the standard deviation of particle size and $\langle d\rangle$ is average value of particle size) for the nanoparticles was 10 %. The DLS size of the 30 and 40 nm SiO_2 nanoparticles was 30.1 ± 0.7 and 39.4 ± 1.2 nm, respectively. The DLS size differed by up to 33 % depending on the detection angles. The DLS size of the 30 nm SiO_2 nanoparticles in aqueous suspension was constant within 15 % of the average value for 1 year. However, the DLS size of 40 nm SiO_2 nanoparticles in aqueous suspension was only constant within 33 % of the average value for 9 months. Based on the time-lapse observation of UV-visible spectra, 30 nm SiO_2 nanoparticles in aqueous suspension were stable for 1 year, while 40 nm SiO_2 nanoparticles in aqueous suspension showed partial sedimentation.

Zeta potential / surface charge

The methods are described in Annex 5 of this dossier (separate document).

Results

The zeta potential was - 81.4 ± 5.3 mV (pH = 8.47) and - 54.6 ± 1.4 mV (pH = 6.22) for 30 and 40 nm SiO_2 nanoparticles in Distilled Water (DW) suspension, respectively. When the suspensions were kept at ambient conditions, the zeta potential of these nanoparticles was maintained for 3 months. However, the zeta potential increased with slopes of 0.14 mV/day and 0.068 mV/day for 30 and 40 nm SiO_2 nanoparticle suspensions, respectively. The pH of the suspensions was stable for 2 weeks but converged to neutral (pH = 7) with increasing storage time.

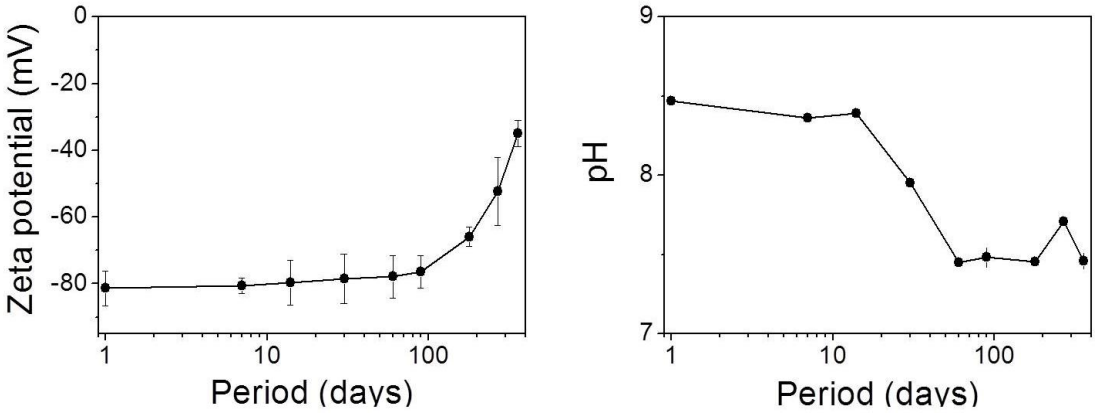


Figure 10. Time dependence of the zeta potential (left) and pH of 30 nm SiO_2 nanoparticles (right) in aqueous suspension

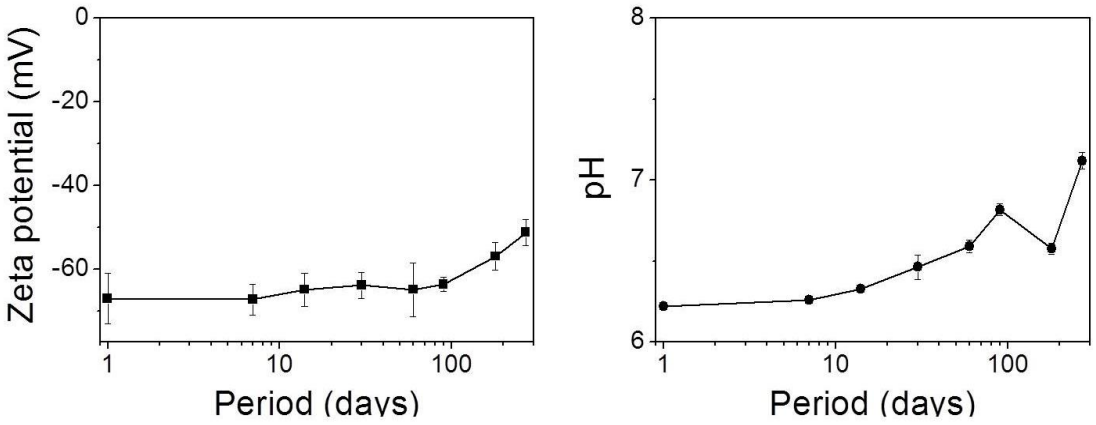


Figure 11. Time dependence of the zeta potential (left) and pH of 40 nm SiO_2 nanoparticles (right) in aqueous suspension.

The zeta potential was thus - 81.4 ± 5.3 mV (pH = 8.47) and - 54.6 ± 1.4 mV (pH = 6.22) for 30 and 40 nm SiO_2 nanoparticles in DW suspension, respectively. The zeta potential was stable up to 3 months but it increased with longer storage time. This is indicating an aggregation of the particles.

Based on the Korean studies of their SiO_2 several papers were published:

1. S.Y. Kwon, Y.-G. Kim, S.H. Lee and J.H. Moon "Uncertainty analysis of measurements of the size of nanoparticles in aqueous solutions using dynamic light scattering" *Metrologia*, 48 417-425 (2011).

2. N.W. Song, K.M. Park, I.-H. Lee, H. Huh, "Uncertainty estimation of nanoparticle size distribution from a finite number of data obtained by microscopic analysis" *Metrologia*, 46(5) 480-488 (2009).

3. Ministry of Education, Science and Technology, Korea, 2010. The Report on "Development of Nano-materials Safety and Characterization Techniques" (KRISS)

4. Ministry of Education, Science and Technology, Korea, 2011. The Report on "Development of Nano-materials Safety and Characterization Techniques" (KRISS).

3. ENVIRONMENTAL FATE AND PATHWAYS

The properties of synthetic amorphous silica have already been reviewed and assessed in several contexts, and several main review reports were identified (OECD 2004, SASSI 2008, and ECETOC 2006); these report all pre-date the WPMN.

In general, SAS is reported to be sparingly soluble in water and values given in the OECD SIDS report range from 15 to 68 mg/l, (OECD 2004). For the NM series the water solubility was determined according to OECD TG 105 to be > 100 mg/l (BIAC unpublished data). The SASSI reports notes that "Given that SAS consists of a relatively unreactive hydrophobic siloxane unit (Si-O-Si) and hydrophilic silanol groups (Si-OH), the solubility of SAS depends on the number of silanol groups per unit surface area (per nm^2). For wet process silica gels, the concentration of silanol groups range from 5 to 8 $SiOH/nm^2$ and for pyrogenic silica, the number is much lower due to the thermal process, ranging from 1.25 to 2.5 $SiOH/nm^2$."

SAS has a vapour pressure of 13.3 hPa at 1732 ºC; its melting point is ca. 1700 ºC (OECD 2004). It is inorganic and fully oxidised, so biodegradation is not relevant. Based on the chemical nature of SiO_2, i.e. the inorganic structure and chemical stability of the Si-O bond, which is highly stable, no photo degradation or chemical degradation is expected.

Based on the properties described above, it is expected that SiO_2 released to the environment will distribute to soil or sediment. One of the main components of any soil or sediment is silicon dioxide (in crystalline or amorphous form). Methods to distinguish SAS NM-20x from naturally occurring amorphous SiO_2 in the environment would be advanced analytical methods requiring for example isotopic labelling of SAS. In case of surface modified silicon dioxide, further investigations might be appropriate.

Under "DETAILS ON CHEMICAL CATEGORY" a description the surface chemistry was given, and the difference between the siloxane group and the silanol group was described, see Figure 3 and Figure 4, as well as different pH dependent reactions with water. This information is very relevant for the environmental fate and pathways.

The SIDS assessment (OECD 2004) states: "*The bioavailable form of synthetic amorphous silica and silicates is the dissolved form which exists exclusively as monosilicic [$Si(OH)_4$] acid under environmental pH. In analogy to the general chemical reaction of weak acids and salts of weak acids with water, the water-soluble fraction of silica acts as a weak acid and, therefore, will tend to lower the pH value, while that of a silicate acts as a base tending to bind protons and, thus, raise the pH value by forming hydroxyl ions (...). But pH shifts which are measurable at high loadings under laboratory conditions are not expected to occur from the anthropogenic deposition in the aquatic environment of synthetic amorphous silica and silicates due to low aquatic releases and sufficient natural buffer capacities.*

Finally, these materials are supposed to combine indistinguishably with the soil layer or sediment due to its chemical similarity with inorganic soil matter.

Dissolved silica can be actively assimilated by some marine and terrestrial organisms as normal natural processes mainly related to structural function."

Based also on the 2004 SIDS assessment, the relevance of testing for the environmental fate and behaviour end-points was evaluated to essentially not be relevant in view of the known information on SiO_2, where the ecotoxicity is linked to the dissolved form. The primary particles of nanostructuted SAS materials NM-20x do not exist on their own, and size of the aggregates of NM-20x would be so large that they would behave more like the bulk material than like primary particles, and it would not, based on size, be likely that they are internalised in an organism. Table 6 gives an overview of available environmental information for NM-20x. In case of surface modified silicon dioxide, further investigations might be appropriate.

Silicon dioxide (in its several naturally occurring forms including crystalline quartz) is one of the most abundant chemical compounds in nature, so *a priori* only little environmental monitoring data on SAS reported in literature was expected, and none was identified.

Korea performed sewage treatment testing with SiO_2 from Sukgyung AT (Korea) and Aldrich (USA), whereas the materials from the NM-series were not tested for this.

Table 6. Environmental information on NM-20x from the JRC repository. Summary of the Environmental Fate Endpoints.

NANOMATERIAL / ENDPOINTS	**SiO_2 Principal Material**	**SiO_2 (Silicon Dioxide (SAS)) Alternate Materials**			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
ENVIRONMENTAL FATE[6]					
1. Dispersion stability in water	A slight sedimentation is observed (DLS measurement, see Rasmussen 2013) during the first hour and then the samples are very stable for the next 16 h (stationary state of Z average and mean count rate).				
2. Biotic degradability	n/a	n/a	n/a	n/a	n/a
• **Ready biodegradability**	n/a	n/a	n/a	n/a	n/a
• **Inherent biodegradability**	n/a	n/a	n/a	n/a	n/a
• **Simulation testing in surface water**	n/a	n/a	n/a	n/a	n/a
• **Soil simulation testing**	n/a	n/a	n/a	n/a	n/a
• **Sediment simulation testing**	n/a	n/a	n/a	n/a	n/a
• **Sewage treatment simulation testing**	n/a	n/a	n/a	n/a	n/a
• **Anaerobic biodegradability**	n/a	n/a	n/a	n/a	n/a
3. Identification of degradation product	n/a	n/a	n/a	n/a	n/a
4. Further testing of degradation products	n/a	n/a	n/a	n/a	n/a
5. Abiotic degradability and fate	n/a	n/a	n/a	n/a	n/a
• **Hydrolysis**	The reactivity of the SAS depends on the degree and the accessibility of the silanol group, especially	See NM-200	The reactivity of the SAS depends on the degree and the accessibility of	See NM-202	See NM-200

NANOMATERIAL / ENDPOINTS	SiO_2 Principal Material	SiO_2 (Silicon Dioxide (SAS)) Alternate Materials			
	Silicon Dioxide (SAS) NM-200 (precipitated)	NM-201 (precipitated)	NM-202 (pyrogenic)	NM-203 (pyrogenic)	NM-204 (precipitated)
	the free ones. NM-200 is synthesized by the precipitation process leading to relatively more silanol groups on the surface than the thermal process. Presence of large amounts of SiOH makes the material more hygroscopic, and it may readily adsorb water molecules from the air.		the silanol group, especially the free ones. NM-202 is synthesized by the thermal process leading to relatively fewer silanol groups than for SAS by the precipitated process. The low presence of silanol groups makes thermal SAS relatively less hygroscopic than precipitated SAS.		
• **Phototransformation**	n/a (Stable in water and Air)	n/a	n/a	n/a	n/a
6. Adsorption-desorption	n/a (OECD TG 106 is not applicable to NMs. Also, the test substance is a major component in soil, thus the method is not meaningful.)				
7. Adsorption to soil or sediment	n/a (OECD TG 106 is not applicable to NMs. Also, the test substance is a major component in soil, thus the method is not meaningful.)				
8. Bioaccumulation potential	n/a (SAS is not lipophilic. SAS is not bio-accumulating due to inherent substance properties.)				
• **Bioconcentration: Flow-through fish test**	n/a				
• **Bioaccumulation is sediment-dwelling Benthic Oligochaetes**	n/a				
9. Other relevant information	n/a				

n/a : not applicable

3.1. Environmental information on SAS nanoparticles tested by Korea (sewage treatment)

Test substances

Nanomaterial name: Silicon dioxide (SiO_2), CAS number: 7631-86-9 (SiO_2)

Remarks: SiO_2 from Aldrich (USA) and Sukgyung AT (Korea)

Methods

Media: De-ionized water and synthetic sewage (Glucose 200 mg/L, yeast extract 10 mg/L, bactopeptone 10 mg/L, $(NH_4)_2SO_4$ 50 mg/L, $K_2H_2PO_4$ 30 mg/L, KH_2PO_4 30 mg/L, $MgSO_4$ 1.8 mg/L, $FeCl_3$ 0.04 mg/L, NaCl 1.4 mg/L, $CaCl_2$ 0.04 mg/L, $CoCl_2$ 0.48 mg/L, $NaHCO_3$ 30 mg/L)

Method/guideline followed: OECD TG 303, "Activated sludge process", OECD TG 106, "Adsorption test"

Type: Batch test

Year (study performed): 2011

GLP: No

Activated sludge: Gimpo sewage treatment plant

Analytical monitoring: ICP (Inductively Coupled Plasma)-Optical Emission Spectrometer (ICP-730 ES, VARIAN, Australia)

Exposure period (duration): 24 h

Doses/concentration levels: 10 mg/L

Test conditions

Dilution water source: De-ionized water

Stock and test solution and how they are prepared: SiO_2 suspension diluted with de-ionized water was exposed to activated sludge

Exposure vessel type: 50 mL polypropylene conical tube

Test temperature: 25℃

Results

Effect of exposure time on fate of SiO_2 in activated sludge process

The adsorption of both Aldrich and Sukgyung AT SiO_2 to activated sludge was affected by the exposure time. The effluent concentration and percent of SiO_2 were progressively decreased with time. The removed percent was increased from 8 h to 24 h exposure time by ca. 0.4% and 0.3% in the case of Aldrich and Sukgyung AT SiO_2, respectively. The results indicate that the retention time of the SiO_2 in activated sludge bioreactor is an important factor for the fate of the SiO_2 in the activated sludge process.

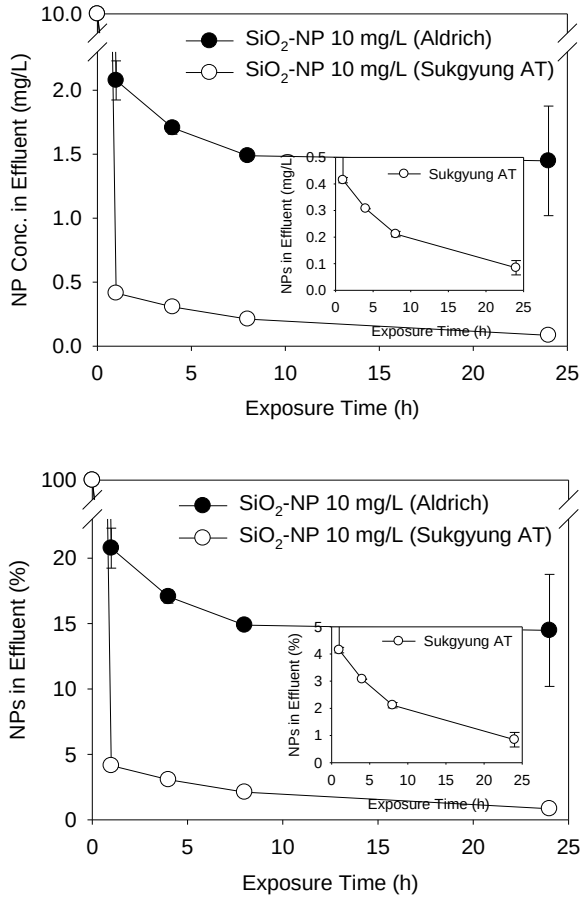


Figure 13. SiO_2 in effluent (deionised water condition); concentration (left) and % = effluent concentration/initial concentration x 100 (right)

Effects of synthetic sewage on SiO_2 concentration in effluent

SiO_2 was added to activated sludge in the presence or absence of synthetic sewage, and the concentrations in the effluent were compared. In the presence of synthetic sewage, more Aldrich SiO_2 (ca. 8%) were adsorbed to the activated sludge as compared with the absence of synthetic sewage (de-ionized water), resulting in a low concentration of SiO_2 (ca. 0.7 mg/L) in the effluent. On the other hand, synthetic sewage induced the reduction of Sukgyung AT SiO_2 adsorption (ca. 2.5%) to the activated sludge. The result suggests that sewage components may affect the fate of SiO_2 in the activated sludge process.

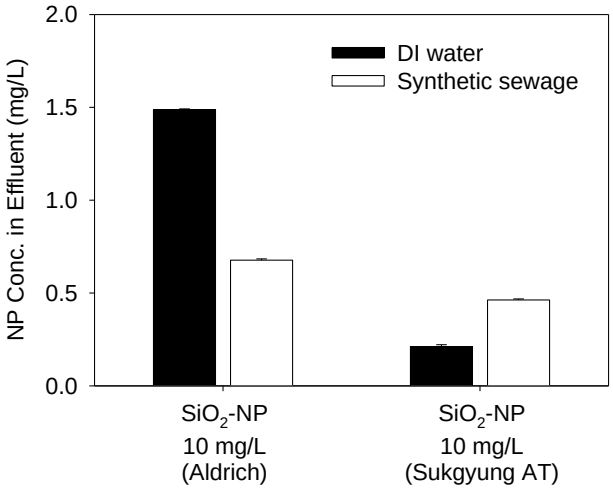


Figure 14. Effect of synthetic sewage on SiO2 concentration in effluent (exposure time: 8 h)

Effects of SiO_2 in effluent water quality

The effluent COD and NH_4-N concentrations of the activated sludge process were monitored after 10 mg/L SiO_2 exposure, and the values were compared with control (i.e. absence of synthetic sludge and absence of SiO_2). There was no significant difference between the COD concentrations of control and both Aldrich and Sukgyung AT SiO_2 exposure, whereas nitrogen concentrations were slightly affected by both Aldrich and Sukgyung AT SiO_2 after 24 hrs.

Table 7. Water quality in the absence and presence of SiO_2 (mg/L)

	Synthetic sewage	8 h			24 h		
		Control	SiO_2 (Aldrich)	SiO_2 (Sukgyung)	Control	SiO_2 (Aldrich)	SiO_2 (Sukgyung)
COD	221 ± 7	116 ± 3	130 ± 39	75 ± 6	89 ± 6	90 ± 4	90 ± 14
NH_4-N	27 ± 1	26 ± 1	21 ± 0	22 ± 0	14 ± 0.4	19 ± 0	17 ± 3

Observations

The fate of both Aldrich and Sukgyung AT SiO_2 was affected by the exposure time and synthetic sewage. 10 mg/L of Aldrich and Sukgyung AT SiO_2 exposure respectively induced 0.7 and 0.5 mg/L of SiO_2 release to the effluent in the synthetic sewage condition for 8 h of exposure time. The effluent COD was not affected by both Aldrich and Sukgyung AT SiO_2 exposure, whereas the nitrogen concentration was slightly changed by both Aldrich and Sukgyung AT SiO_2 after 24 hrs.

References

OECD TG 303 Simulation Test - Aerobic Sewage Treatment

OECD TG 106 Adsorption and Desorption Using a Batch Equilibrium Method.

National Institute of Environmental Research, Ministry of Environment, Korea, 2011. Ecotoxicology and environmental fate for the manufactured nanomaterials.

4. ENVIRONMENTAL TOXICITY

The OECD WPMN testing programme lists the following end-points, see Table 8.

Table 8. End-points for environmental toxicology.

	Environmental toxicology
42	Effects on pelagic species (short/ long term)
43	Effects on sediment species (short/ long term)
44	Effects on soil species (short/ long term)
45	Effect on terrestrial species
46	Effect on micro-organisms
47	Other relevant information

For some of these end points, test results were submitted by BIAC, including a declaration that the substances tested were equivalent to the SAS from the JRC Nanomaterials Repository. However, no characterisation data from those tests were made available to the WPMN to accompany the test results. The test results were originally submitted to support the description of silicon dioxide as an industrial high production volume chemical. The testing predates the WPMN programme. The results are summarised in the table below.

A few studies on environmental effects of SAS were identified as well as several review articles of nanoparticles that included SiO_2. Only one of these studies reported exactly which source of SAS was used. One additional study focuses on *Mytilus* haemocytes instead of the whole organism but can also be considered as an environmental study. Some of the studies have an element of comparison with "bulk" SiO_2 defined as having micron sized grains. In the six identified studies with SAS, the reported characterisation of SAS was limited, and 2 studies are performed using Ludox, which is colloidal SAS. The studies with colloid SiO_2 do not provide data on the characterisation of the composition of the suspension, though addition of a biocide would be expected to ensure shelf life of the commercial product, as biocides have anti-microbial properties.

Table 9. Short term effect on pelagic species

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Main findings**	**Contributor to WPMN Testing Programme**	**Comment**
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1992)**	*Brachydanio rerio*	OECD TG 203 (Fish, Acute Toxicity Test)	6 hours 1000 and 10,000 mg SiO_2/L	After 96 h of exposure all animals were alive and their conditions (swimming behaviour, colour, respiratory function or any other visually observable morphological or behavioural criterion) was equal to that of the control animals.	Data provided by BIAC. Tests performed by TNO Institute of Environmental Sciences Delft (NL) in 1992	
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1992)**	*Brachydanio rerio*	OECD TG 203 (Fish, Acute Toxicity Test)	96 hours 1000 and 10,000 mg SiO_2/L	After 96 h of exposure all animals were alive and their conditions (swimming behaviour, colour, respiratory function or any other visually observable morphological or behavioural criterion) was equal to that of the control animals.	Data provided by BIAC. Tests performed by TNO Institute of Environmental Sciences Delft (NL) in 1992	
Substance declared equivalent to **NM- 202** (pyrogenic) Associated characterisation data not available to the WPMN **(1992)**	*Brachydanio rerio*	OECD TG 203 (Fish, Acute Toxicity Test)	96 hours 1000 and 10,000 mg SiO_2/	After 96 h of exposure all animals were alive and their conditions (swimming behaviour, colour, respiratory function or any other visually observable morphological or behavioural criterion) was equal to that of the control animals	Data provided by BIAC. Tests performed by TNO Institute of Environmental Sciences Delft (NL) in 1992	
Substance declared to be **NM- 203** (pyrogenic) Associated characterisation data not available to the WPMN **(1992)**	*Brachydanio rerio*	OECD TG 203 (Fish, Acute Toxicity Test)	96 hours 1000 and 10,000 mg SiO_2/	After 96 h of exposure all animals were alive and their conditions (swimming behaviour, colour, respiratory function or any other visually observable morphological or behavioural criterion) was equal to that of the control animals	Data provided by BIAC. Tests performed by TNO Institute of Environmental Sciences Delft (NL) in 1992	

Table 10. Short term effect on crustacean species

Material (year of study)	**Test organism / System**	**Method**	**Exposure/ dose**	**Main findings**	**Contributor to WPMN Testing Programme**	**Comment**
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1992)**	*Daphnia magna*	OECD TG 202, 24 h	24 hours 1000 and 10,000 mg SiO_2/L (nominal, loading)	0/40 of the control group were immobilised/dead. 3/40 (7.5 %) and 1/40 (2.5 %) were immobilised /dead at a loading of 1000 and 10000 mg/L respectively. The observed effects were not dose related, and it is likely that they are caused by physical hampering of the test animals.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1992	Test duration 24 h (acc. to the valid guideline of 04 April 1984) instead of 48 h (today) / In one test, the oxygen content was 4.2 mg/L after 24 h, i.e. less than 60 % of saturation (not assumed to have affected the outcome).
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1992)**	*Daphnia magna*	OECD TG 202, 24 h	24 hours 1000 and 10,000 mg SiO_2/L (nominal, loading)	0/40 of the control group were immobilised/dead. 3/40 (7.5 %) and 1/40 (2.5 %) were immobilised/dead at a loading of 1000 and 10000 mg/L respectively. The observed effects were not dose related, and it is likely that they were caused by physical hampering of the test animals.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1992	Test duration 24 h (acc. to the valid guideline of 04 April 1984) instead of 48 h (today). In one test, the oxygen content was 4.2 mg/L after 24 h, i.e. less than 60 % of saturation (not assumed to have affected the outcome).
Substance declared equivalent to **NM- 202** (pyrogenic) Associated characterisation data not available to the WPMN **(1992)**	*Daphnia magna*	OECD TG 202, 24 h	24 hours 1000 mg/L $SiO2$ nominal	Overall 1/40 treated animal was found immobile after 24 h of exposure (2.5 %). Two parallel series using clear or slightly milky solutions of the water soluble fractions were achieved: 0/15 immobile animals (0 %) (assumed to relate to test medium microfiltrated 1.7 µm) 1/25 immobile animals (4 %) (assumed to relate to test medium microfiltrated 1.7 µm and 1.2 µm)	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1992	Test duration 24 h (acc. to the valid guideline of 04 April 1984) instead of 48 h (today) / In one test, the oxygen content was 4.2 mg/L after 24 h, i.e. less than 60 % of saturation (not assumed to have affected the outcome).
Information given in the NM-203 part of the dossier. Associated characterisation data not available to the WPMN **(1992)**.	*Daphnia magna*	OECD TG 202, 24 h	24 hours 1000 mg/L SiO_2 nominal	Overall 1/40 treated animal was found immobile after 24 h of exposure (2.5 %). Two parallel series using clear or slightly milky solutions of the water soluble fractions were achieved: 0/15 immobile animals (0 %) (assumed to relate to test medium microfiltrated 1.7 µm). 1/25 immobile animals (4 %) (assumed to relate to test medium microfiltrated 1.7 µm and 1.2 µm)	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1992	Test duration 24 h (acc. to the valid guideline of 04 April 1984) instead of 48 h (today) / In one test, the oxygen content was 4.2 mg/L after 24 h, i.e. less than 60 % of saturation (not assumed to have affected the outcome).

Summary of published Environmental Toxicology studies

A literature review was performed to identify publications giving environmental toxicology data for SAS. It was not always obvious from the title or abstract of an article whether the SiO_2 studied was SAS or crystalline silica. Thus, some of the identified publications had to be further checked before it was evident which type of SiO_2 was studied. For the sake of completeness, these studies are also listed below in Table 11 (grey background)). Amorphous silica appears to not have been studied much, and only few publications were identified. The table does not include a column for "derived effect value" as none was derived. The identified literature does not always appear very relevant, also due to deficiencies in the reporting of material characterisation, and is included mainly for completeness; it was sometimes difficult to summarise methods and/or Exposure/dose for some of the scientific articles, and this information may not appear in Table 11.

Table 11. Summary of Environmental Toxicology studies published in scientific literature.

Reference	Material / Size	Test Organism / System	Method	Exposure/ dose	Main findings
Effects on pelagic species (short/ long term)					
Fish					
F. Sharif et al. (**2012**)	Mesoporous sílica nanoparticles (Crystalline silica)	zebrafish embryos			Findings not reported here, as a crystalline form of silica was studied.
R. Ramesh et al. (**2013**)	SiO_2 nanoparticles Crystalline silica	zebra fish *(Danio rerio)*			Findings not reported here, as a crystalline form of silica was studied.
Crustaceans					
S.-W. Lee et al. (**2009**)	SiO_2 from Sigma Corp. St. Louis MO, USA. Primary particles of 7nm (fumed) and BET surface area of 644.44 m^2/g and 10nm (porous type) and BET surface area of 349.71 m^2/g	*Daphnia Magna and Chironomus riparius*	OECD TGs 202 and 211	1 mg/L	*D. magna* and the larva of *C. riparius* were exposed to nanosilicas, one precipitated (primary particle size 10nm) and one pyrogenic silica (primary particle size 7nm) (Lee, 2008). No genotoxic effects, measured as DNA strand breaks by the comet assay, were observed on these two species. Exposure to SiO_2 had no genotoxic effect on either species. SiO_2 did not seem to affect DNA integrity whereas the mortality of the SiO_2 exposed *D. Magna* and *C. riparius* increased. mortality *D. magna* *C. riparius* 7 nm : 10% ± 8% 5% ± 4 10 nm : 15% ± 4% 20% ± 0

					Control 5% ± 4.08 0
M. Casado et al. (**2013**)	SiO_2 from Kisker Biotech GmbH (amorphous) Amorphous and mono-disperse silica nanoparticles of 50 nm and 100 nm. Plain silica NPs and green fluorescently labelled silica NPs were tested	*Daphnia Magna and* *Thamnocephalus platyurus*	OECD TG 202 Kit from SDI Europe	0.1 to 1000 µg/mL 0.1 to 1000 µg/mL	Plain and fluorescently labelled silica NPs showed no significant toxicity in any of the acute ecotoxicity tests performed on the different organisms for both diameters. Such a response may be expected as amorphous silica NPs are known for their low toxicity (Barnes et al. 2008; Rabolli et al. 2010), indicating their suitability as a good negative control for NP exposure. In the case of the cytotoxicity testing, both assays indicate a low dose and exposure time dependent response a slightly larger effect being observed for the 50 nm than the 100 nm silica NPs in both assays.
J.Mankiewicz-Boczek et al. (**2009**)	Ludox® SiO_2 CL-X nanoparticles (21 nm)	*Thamnocephalus Platyurus* *Heterocypris Incongruens*	Thamnotoxkit F™ Ostracodtoxkit F test.	50 to 1000 mg/L 300 to 1000 mg/L	Silica LUDOX® CL-X was not toxic to the tested crustaceans at the maximum concentration tested (1,000 mg/l).
Algae					
K. Fujiwara et al. (**2008**)	Silica nano-particles of 5, 26 and 78 nm from Shokubai Kasei Industrial Co.	*Chlorella kessleri*			No characterization data of the SiO_2 are provided. The conclusions state ".. . Other amorphous nano-silica should be inspected with regard to bio-toxicity. …" which indicate that the silica nanoparticles investigated are amorphous. The investigated silica nanoparticles show size dependent toxicity ("appearance of some amorphous structures on the cell, obstruction of cell division, and faint of chlorophyll colour").
K. van Hoecke et al. (**2008**)	Ludox® LS and TM 40 silica nanoparticles (12.5 nm and 27 nm) The specific surface areas of the particles are 236 and 135 m^2/g SiO2 for LUDOX LS and TM40, respectively. The particles in LUDOX are monodispersed, discrete uniform spheres of silica that have no internal	*Pseudokirchneriella subcapitata*	OECD TG 201	2.2 to 460 mg/L.	Electron microscopy images of exposed algae cells did not provide evidence for the internalization of nanoparticles in the algal cells. No significant changes in shape or cell morphology were noted. However, considerable adsorption of the NPs to the outer surface of the cells was observed. When the results of the algal growth–inhibition test using $Na_2SiO_3 \cdot 5H_2O$ were compared to the concentrations of reactive silica in the nanoparticle suspensions, it could be concluded that reactive silica (4.14 mg/L SiO_2) could not be responsible for the observed toxic effects of the LUDOX suspensions. Hence, it is clear the observed adverse effects to *P. subcapitata* were due to the SiO_2 nanoparticles. On a mass basis, the smaller particles were more toxic, although the difference in toxicity disappeared when concentration was expressed as a surface area. The total

	surface area or detectable crystallinity.				surface area may be a better measurand for the toxicity of SiO_2 nanoparticles. The paper does not discuss possible effects from the dispersant and any antimicrobial agents in the colloid.
K. van Hoecke et al. (**2011**)	Ludox® SiO_2 CL-X nanoparticles (22 nm) specific surface area 102 m^2/g	*Pseudokirchneriella subcapitata*	OECD TG 201	4.6 to 1000 mg/l	*P. subcapitata* (green algae) was exposed to silica, Ludox®, which is a colloid amorphous silica. SiO_2 NPs were not toxic at pH 6.0 up to a conc. of 220 mg/l. Chlorophyll analysis of algae exposed to the SiO_2 NPs indicated a much more severe effect on algal growth rate compared to the cell count based data. In fact, at the highest test concentration, the algal cells almost completely lacked chlorophyll. It turned out that nanoparticles toxicity can be strongly pH dependent (from pH 6.8). These results suggest that breakdown of chlorophyll and/or chlorophyll synthesis inhibition was an important aspect of the mechanistic toxic response induced by the SiO_2 NPs. In fact, at the highest test concentration, the algal cells almost completely lacked chlorophyll. The paper does not discuss possible effects from the dispersant and any antimicrobial agents in the colloid.
C. Wei et al. (**2010**)	Silica nanoparticles from Sigma Aldrich (10-20 nm) with a purity of 99.5 %	*Scenedesmus obliquus*		25 to 200 mg/L.	*S. obliquus* was exposed to nanosilica particles from Sigma Aldrich and bulk silica from Shanghai Chemical Reagent Company of China. SEM micrographs of both silicas are provided, but no further characterisation; the SEM micrograph of bulk SiO_2 shows a very angular structure which could be crystalline – this is not discussed in the paper. The algal growth rate decreased as a function of the following exposure concentration (50, 100, and 200 mg/L) and time (48, 72, and 96 h). These results indicate that there is some degree of toxicity to S. obliquus when exposed to SiO_2 NPs in the aquatic environment but the inhibition rate of the highest concentration groups did not reach 50%. The algal cells did not change morphologically when observed under the optical microscope, which was consistent with the phenomenon observed by Van Hoecke et al. (2008)
M. Casado et al. (**2013**)	SiO_2 from Kisker Biotech GmbH (amorphous) Amorphous and Monodisperse silica nanoparticles of 50 nm and 100 nm. Plain silica NPs and green fluorescently labelled silica NPs were tested	*Pseudokirchneriella subcapitata*	OECD TG 201	100 µg/mL	For both diameters plain and fluorescently labelled silica NPs induced no significant toxicity in any of the acute ecotoxicity tests performed on the different organisms.
J. Ji et al.	SIO_2 from Zhejiang	*Chlorella sp.*	.	0.5 to 1000	No significant toxicity was observed for nano-SiO_2 with concentration up to 1000 mg/L

(**2011**)	Hongsheng Material Technology Co. 4 types of nanoparticles were tested: - SS1 (20-50 nm) spherical with a specific surface area 102 m^2/g - DS1 (20-50 nm) spherical with a specific surface area 221 m^2/g - SP1 (20-50 nm) spherical with a specific surface area 570 m^2/g - DP1 (20-50 nm) spherical with a specific surface area 675 m^2/g			mg/L.	during the 6 days except that SS1 and SP1 inhibited the algal growth by ca. 20% ($p < 0.05$) at the 2nd day.
D. M. Metzler et al. (**2012**)	4 types of nanomaterials from Degussa Corp: were tested: (produced by continuous flame hydrolysis) Aerosil 90 (35.6 nm) l with a specific surface area 76.5 m^2/g Aerosil 130 (26 nm) with a specific surface area 105.1 m^2/g Aerosil 200 (14.3 nm) with a specific surface area 190.6 m^2/g Aerosil 300 (9.6 nm) with a specific surface area 248.8 m^2/g	*Pseudokirchneriella subcapitata*		100 and 1000 mg/L.	Van Hoecke et al. found that SiO_2 at particle size of 12.5 and 27.0 nm had a 72 h specific growth rate EC_{20} of 20.0 ± 5.0 and 28.8 ± 3.2mg/L, respectively Of the NP concentrations used in the Van Hoecke et al. study, the highest concentration of dissolved Si was 4.1 mg/ L at pH 7.5. The measured dissolved Si at EC_{20} in the present study was 234 mg/L, which was adequate to affect the growth of algae. Therefore, dissolved Si was not considered a major actor in SiO_2 toxicity. The difference in EC values between this work and that of Van Hoecke et al. could be due to different initial algal densities. In this study, the initial cell density was 106 and Van Hoecke et al. used 105 cell/mL. SiO_2 did not play a major role in the growth of P. subcapitata over the concentration range tested in our work, which agreed with Ji et al. who reported little effect on Chlorella sp. Growth at SiO_2 concentration of 1000 mg/L in the size range of 20–50 nm. SiO_2 caused an increase in lipid peroxidation. An increase in SiO2 caused an increased average normalized specific lipid peroxidation,. Although not considered a photocatalyst, SiO_2 has been observed to produce similar photosensitive effects, including increased ROS levels and reduced glutathione levels, as a photocatalyst. SiO_2 affected the chlorophyll content of the algal cells. Low NP concentration increased the concentration of chlorophyll, whereas high NP concentration decreased it. Results showed that limited light availability can affect the chlorophyll content in algae. The reduction in light availability would encourage the algae to produce more chlorophyll

					per cell. At low concentrations the algae will attempt to overcome the decreased light availability.
N. Oya San et al. (**2014**)	Silica Nanoparticles produced by laser ablation (38–190 nm)	*Chlorella vulgaris.*			Silica NPs produced by laser ablation increased the growth of C. vulgaris. Data on the concentration tested are missing, as are graphs for the concentration-effect curves, no TEM pictures are available for showing if morphology is affected and if the nanoparticles were internalised in the cells.
Effects on sediment species (short/ long term)					
L. Canesi et al. (**2010**)	Aerosil 200 (12 nm) with a specific surface area 205 m^2/g from Degussa Evonik.	*Mytilus galloprovincialis*		0.05 to 5 mg/mL	Characterisation data provided. No mortality was observed in any condition of exposure. The nanomaterial did not induce lysosomal membrane destabilisation. Lysosomal lipofuscin and catalase activity were increased; whereas GST (glutathione-S-transferase) activity was not. The digestive gland appears to be the main target for nanoparticle toxicity.
L. Canesi et al. (**2010**)	Aerosil200 (12 nm) with a specific surface area 205 m^2/g from Degussa Evonik.	*Mytilus hemocytes*	In vitro	1, 5 and 10 µg/mL	Characterisation data provided. Test concentrations of 1, 5 and 10 µg/mL did not induce significant cytotoxicity, but stimulated lysozyme release, oxidative burst and NO production.
Effects on soil species (short/ long term)					
A. Pluskota et al. (**2009**)	Amorphous silica nano-particles 50nm from Kisker, Germany (both labelled and unlabelled) Amorphous bulk silica particles 500 nm from Kisker, Germany	*caenorhabditis elegans*		Concentrations of 0.25mg/ml – 7.1 µg/cm^2 0.5mg/ml – 14.2 µg/cm^2 2.5mg/ml – 71 µg/cm^2 5 mg/ml– 142 µg/cm^2 in aqueous suspension. 50 µl applied to bacterial lawn.	Fluorescently labelled nanoparticles are efficiently taken up by *Caenorhabditis elegans* during feeding, and translocate to primary organs such as epithelial cells of the intestine, as well as secondary organs belonging to the reproductive tract. The life span of nanoparticle-fed worms remained unchanged whereas a reduction of progeny production was observed in silica nanoparticle exposed worms versus untreated controls. It is suggested that silica-nanoparticles induce an age-related degeneration of reproductive organs.
Effects on terrestrial species (short/ long term)					
Y. Liang et al. (**2007**)	Silicon	Higher plants Review			It is suggested that Si should be considered an essential element for higher plants. Silicon is known to effectively mitigate various abiotic stresses such as manganese,

					aluminium and heavy metal toxicity and salinity, drought, chilling and freezing stress. The review is about the mechanisms of this alleviation. The publication did not look into uptake of nano-silica.
H.A. Currie and C.C. Perry (**2007**)	Silica	plants Review			Currie et al. (2007) observed that plants take up silicic acid, and that the ".. presence of Si in plants has been found to alleviate many abiotic and biotic stresses, leading to the incorporation of silicates into many fertilizers…". The publication did not look into uptake of nano-silica.
M. H. Siddiqui et al. (**2014**)	Nano-SiO_2. Aerosil 200 (12 nm) with a specific surface area 200 m^2/g from Degussa Evonik.	Tomato (*Lycopersicum esculentum* Mill. cv Super Strain B)		2-10 g/L	The present experiment was conducted to test the beneficial effects of silicon dioxide ($nSiO_2$: size 12 nm) on the seed germination of tomato (Lycopersicum esculentum Mill. cv Super Strain B). Application of $nSiO_2$ significantly enhanced the characteristics of seed germination. It improved percent seed germination, mean germination time, seed germination index, seed vigour index, seedling fresh weight and dry weight. Therefore, it is very clear that $nSiO_2$ has a positive significant impact on the seed germination potential.
V. Shah et al.(**2009**)	3-aminopropyl functionalized silica nanoparticles from Sigma Aldrich chemical Co.	Lettuce seeds		0.013% and 0.066% (w/w)	Results show a statistically insignificant influence of the nanoparticles in the soil on the number of colony forming units, peak areas of methyl ester of fatty acids in the FAME profile or on the total soil community metabolic fingerprint ($P>0.05$). The nanoparticles tested in the study influenced the growth of lettuce seeds as measured through shoot/root ratios of the germinated plant ($P<0.05$).
M. Kalteh et al. (**2014**)	The particles were produced from rough rice. One gram of silicium particles with 7 nm diameter has absorption surface equal to 400 m^2	Basil (*ocimum basilicum*)		10 ml silica nanoparticles was dissolved in 1 L of distilled water and sprayed on plants.	Silica nanoparticles were found to reduce the pollution effects on Basil originating from salinity.
Effect on micro-organisms					
L.K. Adams et al. (2006)	Nanoscale SiO_2 14 nm, 930 nm and 60 µm particles from Sigma Aldrich	*B. subtilis* *E. coli*			The publication is unclear on the identity of the tested SiO_2 (characterization data missing, production method missing, no information on whether crystalline or amorphous, literature references to both crystalline and amorphous SiO_2). Although this article stated that the effects of particles could not be effectively measured in this study, it also noted a toxicity displayed by nanosized SiO_2 toward *B. subtilis*. The information was deemed of no further use for the report.
W. Jiang et	Ludox Cl, 20nm from	*B. subtilis*			Nano-SiO_2 showed highly significant ($p<0.01$) toxicity towards all three bacteria. The

al. (**2009**)	Sigma Aldrich and micro-sized particles from Fisher Scientific Co.	*E. coli* *P. fluorescens*			possible effects from antimicrobial agent(s) in the colloid are not discussed. Microsized particle SiO_2 showed no toxicity towards the three bacteria types.
C. Garcia-Saucedo et al. (**2011**)	SiO_2 nanoparticles 10- 20 nm from American Elements	*Saccharomyces cerevisiae*			Garcia-Saucedo et al. (2011) exposed the yeast *S. cerevisae* to silica nanoparticles. They conclude "Taken as a whole the results of this study demonstrate that nano-sized oxides evaluated … SiO_2, … are not expected to be toxic to *S. cerevisiae* cells at environmentally relevant concentrations."
Other information/background information					
K. Yang et al. (**2009**)	Nanosized Inorganic Oxides	Humic Acid			The interaction of humic acid with nanosized inorganic oxides, including SiO_2, was investigated (Yang et al., 2008). The SiO_2 is described as S or P form and it is not clear whether it is crystalline or not as the characterisation data are limited. According to the results, humic acid does not adsorb to SiO_2. The information was deemed of no further use for the report
L. Reijnders (**2009**)	SiO_2 nanoparticles	release from nanocomposites			Noting that nanomaterials may be hazardous and may be released from composites this article review how to make safer composites
B. Nowack and T.D. Bucheli (**2007**)	nanoparticles	review			These review present effects of nanoparticles (natural and manufacturers) on the environment. Very few studies about SAS nanoparticles were reported.
M. Cassado et al. (**2013**)	SiO_2 from Kisker Biotech GmbH (amorphous) Amorphous and Monodisperse silica nanoparticles of 50 nm and 100 nm. Plain silica NPs and green fluorescently labelled silica NPs were tested	*Vibrio Fischeri*	SDI Europe (MIcrotox test)	0.1 to 1000 µg/mL	Plain and fluorescently labelled silica NPs showed no significant toxicity in any of the acute ecotoxicity tests performed on the different organisms for both diameters

5. TOXICOLOGICAL INFORMATION

Several studies have been identified reporting results of in vivo tests relevant for mammalian toxicology, emphasis given to studying inhalation effects to understand pulmonary toxicity. While the pulmonary toxicity effects of crystalline silica is abundantly described due to the wide occupational exposure and association with severe pulmonary pathologies like silicosis (Hamilton, Jr. *et al.*, 2008; Iyer *et al.*, 1996), the toxicity of SAS particles (micro-sized or nano-sized particles) has not been widely studied (Napierska *et al.*, 2009; Merget *et al.*, 2002b; Cho *et al.*, 2007).

According to current knowledge, the inhalation constitutes a major pathway for SAS to enter into the human body. Regarding epidemiological data on SAS, with the exception of a few case reports with poorly described exposure scenarios, there is no evidence of a fibrogenic effect of SAS to the human lung. As the available information on humans is not sufficient to exclude a fibrogenic effect of SAS in exposed workers, further epidemiological evidence should be obtained (Merget *et al.*, 2002). The digestive absorption of mineral compounds like SAS depends on their solubility and on the ingested amount. Ingested dust is stored in the intestinal mucous membrane. The non-assimilated fraction is eliminated directly in the faeces. The long-term retention is expected to be weak. However studies specific to SAS are lacking. The penetration of SAS in the organism by the dermal route seems also minimal but this point is only rarely discussed in any study.

In vivo animal studies show that SAS induces a strong inflammatory response in the lungs (Arts *et al.*, 2007; Cho *et al.*, 2007), the response being even higher with ultrafine particles of SAS (Kaewamatawong *et al.*, 2005), but the effect is transient and reversible after a 3-month recovery period in rats (Arts *et al.*, 2007) or even earlier in mice (Cho *et al.*, 2007). The recruitment number of leukocytes and neutrophils concentrations in bronchoalveolar lavage fluid seems to be somewhat lower, and may decrease faster than for quartz (Ernst *et al.*, 2002; Chen *et al.*, 2004). The transient effect can be attributed to the rapid clearance of amorphous silica from the lungs to other organs (Nemmar *et al.*, 2006). However some studies describe a minor persistent interstitial collagen deposition (Merget *et al.*, 2002) or some remaining histopathological changes ((Arts *et al.*, 2007).

An animal inhalation study using SAS encapsulated TiO_2 has also shown reversible pulmonary inflammation, emphysema and alveolar hyperinflation (Warheit *et al.*, 2006), although these data are not further used as this report focus on pure SAS particles.

SAS has been approved for use as a food additive (E 551), but no studies were identified that provide a thorough characterization of the fate of ingested SAS.

For protocols and procedures used in the Nanogenotox project for the genotoxicity testing, these are published at the project's homepage, http://www.nanogenotox.eu/.

5.1 Toxicokinetics, Metabolism and Distribution

- In vitro studies

No in vitro toxicokinetics data is available for the NM-series.

- In vivo studies

BIAC provided data for toxico-kinetics via the inhalation route, see Table 12, which contains an overview of the Toxicokinetics (Inhalation) study summaries provided. Some of the data was generated before the WPMN set up its testing programme, and for those studies it is stated that the material tested is equivalent to the NM-series.

Table 13 gives an overview of the toxicokinetics (oral route) study summaries provided. The Nanogenotox project generated some of the data testing the NM-seriess; BIAC provided data pre-dating the WPMN testing programme stating that the testmaterial is equivalent to the NM-series.

Table 14 provides an overview of toxicokinetics (intravenous route) study summaries provided. The data was generated in the Nanogenotox project using the the NM-series materials.

Table 12. Overview of study summaries provided for Toxicokinetics (Inhalation)

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Main findings**	**Contributor to WPMN Testing Programme**	**Comment**
NM 200 (precipitated) **(2014)**	Rats (Wistar, male)	Inhalation, Subchronic Inhalation toxicity study, 90d, acc. OECD TG 417	90 days/ 6h/d, 5d/week with effectively 65 exposure days 1,2.5 and 5 mg/m^3	Calculation of retention half-times using first order kinetics comes out with 32, 31 and 28 days in the low, mid and high dose groups respectively. This demonstrates an evident dissolution effect, in addition to the physiological particle clearance effect	Data provided by BIAC. Tests performed by Fraunhofer ITEM - Institute for Toxicology and Experimental Medicine Hannover, Germany in 2011	
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar, male and female	Inhalation Subchronic Inhalation Toxicity: 90-day Study (OECD TG 413)	90 days 35 mg/m^3 Sampling: 1, 13, 29, 39, and 52 weeks post exposure	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 decreased during recovery.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987	Special modifications compared to standard study: Focus upon lung, respiratory tract, and regional lymph nodes. Post-exposure recovery period up to one year
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar, male and female)	Inhalation Subchronic Inhalation Toxicity: 90-day Study (OECD TG 413)	90 days 35 mg/m^3 Sampling: 1, 13, 29, 39, and 52 weeks post exposure	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 decreased during recovery	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987	Special modifications compared to standard study: Focus upon lung, respiratory tract, and regional lymph nodes. Post-exposure recovery period up to one

						year
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1969)**	Rat (Sprague-Dawley female)	Inhalation	6 and 18 weeks and 12 months (Interim kill after 6 and 18 weeks) 5 h/d, 5x/wk initially, but later (time not stated) weekly frequency reduced to 2-3x/wk because suppurative bronchitis and severe inflammation caused losses. Post exposure (recovery period): 5 months	After12 months exposure, about 1 % of administered total respirable dust was estimated to be still retained in the lung. The increase in lung deposition was low from 18 weeks to 12 months of exposure (18 wk 1.2 mg SiO_2, 12 months: 1.37 mg SiO_2). Mediastinal lymph nodes contained about 0.13 mg SiO_2 after 12 months. After 5 months post-exposure, mean levels of SiO_2 were 0.16 mg/lung and 0.047 mg/lymph node, i.e. a reduction at some 88 % in the lung and more than 50 % in the lymph nodes. PATHOLOGY: Microscopically visible small dust foci could be observed under the pulmonary pleura, mediastinal lymph nodes were moderately enlarged. In the interior of alveoles, numerous macrophages accumulated, partially normal, partially destroyed, associated with deposition of cell debris ("desquamation catarrh") Perivascular and peribronchiolar small dust foci of macrophages, associated with mild and moderate formation of connective tissue (ranke as grade I to II, based on a ranking systemacc. to Belt&King). In the alveolar septa the collagen formation was increased. In some cases collagenic fibrosis was detected.There were no signs of typical silicosis. In the mediastinal lymph nodes, foci and clusters of phagocytes, partially normal, partially showing decay, were observed.	Data provided by BIAC. Tests performed by Institute of Hygiene and Occupational Medicine, Essen (DE) in 1969	
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar male and female)	Inhalation Subchronic Inhalation Toxicity: 90-day Study (OECD TG 413)	90 days 1.3, 5.9 or 31 mg/m^3 (mean analytical values) Recovery period: 1, 13, 29, 39, and 52 weeks	Silica could be detected in lungs only in relatively small amounts one week after the end of the exposure period, on average 0.2 mg in all animals of the 30-mg groups, in 10 male and 7 female rats of the 6-mg groups, and in 3 animals of each in the 1-mg groups. Only in one untreated male animal, a low level of Si was detected. Only one male exposed to 30 mg/m^3 showed a small amount of silica in the regional lymph node. No significant increased Si levels were observed at any other recovery interval.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987	Special modifications compared to standard study: Focus upon lung, respiratory tract, and regional lymph nodes. Post-exposure recovery period up to one

						year
Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar male and female)	Inhalation Subchronic Inhalation Toxicity: 90-day Study (OECD TG 413)	90 days 1.3, 5.9 or 31 mg/m^3 (mean analytical values) Recovery period: 1, 13, 29, 39, and 52 weeks	Silica could be detected in lungs only in relatively small amounts one week after the end of the exposure period, on average 0.2 mg in all animals of the 30-mg groups, in 10 male and 7 female rats of the 6-mg groups, and in 3 animals of each in the 1-mg groups. Only in one untreated male animal, a low level of Si was detected. Only one male exposed to 30 mg/m^3 showed a small amount of silica in the regional lymph node. No significant increased Si levels were observed at any other recovery interval	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987	Special modifications compared to standard study: Focus upon lung, respiratory tract, and regional lymph nodes. Post-exposure recovery period up to one year

Table 13. Overview of study summaries provided for Toxicokinetics (oral route) *(no comments were given so comment column is not included)*

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Main findings**	**Contributor to WPMN Testing Programme**
NM-200 (precipitated) **(2013)**	Rat (Sprague-Dawley. male and female)	Oral (gavage) Sampled organs: liver, spleen, GI tract (small intestine), mesenteric lymph nodes	20 mg/kg bw/d (male and female) Cumulative dose: 100 mg/kg bw Administration: repeated (on 5 consecutive days, day 1-5) Sampling time: day 6 and day 14 Blood sampling: day 5 t=30 min, t=60 min, t=2 h, t=4 h, t= 8 h, day 6	Bioaccumulation negligible or absent of NM200 following repeated oral administration of 20 mg/ml Very low levels in the liver and spleen (< 2 mg/kg organ weight) near the LOQ and LOD indicating a very low absorption from the gastro-intestinal tract.	Data provided by Nanogenotox. Study performed by ISS (I) in 2013
Substance declared equivalent to **NM-202** Associated characterisation data not available to the WPMN **(1969)**	Rat (Sprague-Dawley, female)	Oral (gavage)	100 mg/animal (approx. 500 mg/kg) 20 administrations	In 20 rats receiving 20 daily oral doses of 100 mg Silica per animal (about 500 mg/kg bw) each, tissue values apparently were very slightly increased in liver and kidney: in liver 4.2 µg (control value 1.8 µg), in the spleen 5.5 µg (7.2 µg) and in the kidneys 14.2 µg (7.8 µg).	Data provided by BIAC. Tests performed by Institut für Hygiene und Arbeits-medizin, Klinikum Essen (DE) in 1969
NM-203	Rat	Oral (gavage)	20 mg/kg bw/d (male and female)	Bioaccumulation negligible or absent of NM203	Data provided by

(pyrogenic) **(2013)**	(Sprague-Dawley. male and female)	Sampled organs: liver, spleen, GI tract (small intestine), mesenteric lymph nodes	Cumulative dose: 100 mg/kg bw Administration: repeated (on 5 consecutive days, day 1-5) Sampling time: day 6 and day 14 Blood sampling: day 5 t=30 min, t=60 min, t=2 h, t=4 h, t= 8 h, day 6	following repeated oral administration of 20 mg/kg Very low levels in the liver and spleen (< 2 mg/kg organ weight) near the LOQ and LOD indicating a very low absorption from the gastro-intestinal tract.	Nanogenotox. Study performed by ISS (I) in 2013

Table 14. Overview of study summaries provided for Toxicokinetics (Intravenous) *(no comments were given so comment column is not included)*

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Main findings**	**Contributor to WPMN Testing Programme**
NM-200 (precipitated) **(2013)**	Rat (Sprague-Dawley. male and female)	Intravenous Tissues sampled: liver, spleen, kidneys, heart, lungs, brain, testes/ovaries	Single (day 1) or repeated (5 consecutive days) 20 mg/kg bw/d (male and female) Cumulative dose: 100 mg/kg bw Administration: Single (day 1) or repeated (on 5 consecutive days, day 1-5) Sampling time: - Single admin: day 2 and day 90 - Repeated admin: day 6, 14, 30 and 90 (day 6 and 90 for female) Blood sampling: - single and repeated admin (day 1): t=5, t=10, t=20, t=30, t=60 min, t=2 h, t=4h, t=8h, t=24h	Bioaccumulation of Si in liver >spleen, lungs at day 2 and 6 which decreases at or below the limit of quantification at day 90 following single dose whereas it is still above the control in liver and spleen following repeated administrations Single administration: liver >spleen, lungs Decrease in Si level at or below LOQ in all organs at 90 days Repeated administrations: day 6: liver> spleen and lungs. Si >LOQ in all other organs At day 90, Si still >LOQ in liver and spleen	Data provided by Nanogenotox. Study performed by ISS (Italy) in 2013
NM-203 (pyrogenic) **(2013)**	Rat (Sprague-Dawley. male and female)	Intravenous Tissues sampled: liver, spleen, kidneys, heart, lungs, brain, testes/ovaries	Single (day 1) or repeated (5 consecutive days) 20 mg/kg bw/d (male and female) Cumulative dose: 100 mg/kg bw	Single administration: day 6, spleen >liver, lungs. Levels of Si abovebackground in heart, kidney, testis day 90: The level of Si had decreased significantly, but was still higher than control in spleen and liver Repeated administrations: day 6: concentrations very high liver> spleen and lungs. Si >LOQ in all other organs	Data provided by Nanogenotox. Study performed by ISS (Italy) in 2013

				At day 90, The level of Si had decreased significantly, but was still higher than the control in liver and spleen	

5.2 Acute toxicity

The tables below, Table 15 and Table 16, summarise the results for acute toxicity; Table 15 gives data for the alternate material supplied by Japan and Table 16 summarises the information from scientific literature of toxic response to a single dose of test material. Table 17 provides an overview of acute toxicity study summaries provided by BIAC. The data concerns the following exposure routes: inhalation, oral and dermal. The data is generated before the WPMN set up its testing programme, and it is stated that the material tested is equivalent to the NM-series.

Table 15. Data supplied by Japan on their alternate material

TEST ITEM	Nanomaterial Information	TEST ORGANISATION	TEST METHOD(S)	TEST RESULTS/ COMMENTS	ANY ISSUES IDENTIFIED
***In Vitro* tests**	Two different Amorphous SiO_2 nanoparticles: **UFP-80** primary particle size: 34 nm (measurement method not stated) purity: > 99.5 % from Denki Kagaku Kogyo Kabushiki Kaisha (Japan) http://www.denka.co.jp/eng/denzai/product/25.html **Nanotek** -primary particle size: 25 nm (TEM, average) -amorphous, -spherical shape, -specific surface area (BET): 86.0m^2/g, - purity: 99.9 % - from C. I. Kasei Co. Ltd. (Japan). Physical Vapor Synthesis (PVS) method http://www.cik.co.jp/product/nanotek/english/	Japan/AIST http://www.aist-riss.jp/projects/nedo-nanorisk/rd/iwahashi2009_e.html Contact: t-igarashi@aist.go.jp	Ten different cell lines including A549, HaCaT, and THP-1. Cell viability, oxidative stress, DNA injury, colony forming ability, gene expression of cytokine and apoptosis.	SiO_2 nanoparticles induced oxidative stress in cultured cells. The intracellular ROS level was elevated by SiO_2 exposure. Subsequently, cell viability was decreased. The MTT activity was slightly decreased (50 % of untreated cells) at conc. of approx. 50 μg/ml for 24 h exposure. Activity of apoptosis related enzyme caspase-3 was increased by 24 h exposure.	

Single dose toxicity

A/ *In vivo* Studies

Table 16. Summary of information from scientific literature. *(The table does not include a column for "Derived effect value" as none was derived.)*

Reference	Material / Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Pulmonary route					
Arts, JHE, et al, **2007**	Zeosil 45 Syloid 74 Cal-O-Sil M5	Wistar rats	Inhalation	6 h/day for 5 consecutive days 1, 5 or 25 mg/m^3 Sampling at day 6, 1 month and 3 months following exposure	Transient and reversible elevation of biomarkers of cytotoxicity in BAL fluids, during the 3 months recovery period. Slight histopathological lung changes at the higher exposure levels.
Chen et al. **2004**	Unspecified nanosized silica (SAS?) provided by Zhoushan Mingri Nanomaterial Limited Company (Zhejiang, China)	Wistar rats	Inhalation	40 min/day for 4 weeks 24.1 mg/m^3	The fibrogenic effect of nanosized SiO_2 might be milder than that of microsized SiO_2 in rats.
Sayes et al. **2007**	Zeofree 80	Male Crl :CD(SD)IGS BR Rats	Intratracheal instillation	Single doses: 1 or 5 mg/kg Sampling at 24 h; 1 week; 1 month; 3 months post exposure	Pulmonary instilled SAS produced reversible and transient inflammatory response in rats.
Cho WS, **2007**	14 nm SAS particles from Sigma Aldrich	A/J mice	Intratracheal instillation	Single doses: 2, 10 and 50 mg/kg Sampling at 24 h, and 1, 4 and 14 weeks following exposure	Increase in the lung weights, in total BAL cells and in several pro inflammatory mediators during the early stages. No changes were detected after week 1 or 4. Instillation of SAS induced transient, but very severe lung inflammation.
Kaewamatawong et al., **2005**	14 nm (UF SAS) / 213 nm (Fine SAS) (manufacturer's	ICR mice	Intratracheal instillation	Single dose: 3 mg Sampling 30 min to 24 h post exposure	Ultrafine SAS causes more lung inflammation and tissue damages than Fine SAS Between 30 min and 24 hours post exposure

	specifications)				
Kaewamatawong et al., **2006**	14nm (Fuso Chemical Co.) Surface specific area 194 m^2/g (manufacturer specifications)	ICR mice (male)	Intratracheal instillation	Single doses: 0.3; 3; 10; 30 and 100 µg Sampling 3 days postexposure	Transient acute moderate lung inflammation and tissue damage. Oxidative stress and apoptosis may underlie the lung tissue injury induction. This study demonstrated the pulmonary biological and pathological responses after intratracheal instillation of low dose of SiO_2 NPs in mice during the acute and subacute stages. Low dose of SiO_2 NP produced moderate inflammation and tissue damage on the lungs of mice during the acute period, but these responses were not sustained through a 30-day period after instillation and almost recovery at the subacute stage. Furthermore, SiO_2 NP can induce oxidative damage and apoptosis, which may be underlying causes of the lung tissue injury. The data from the dose and time responses in this study may be useful in predicting the acute and subacute effects of SiO_2 NP on lungs.
Choi, M et al. **2008**	Unspecified 14 nm SAS from Sigma Aldrich	A/J mice	Intratracheal instillation	Single doses: 2; 10; 50 mg/kg Sampling : 24 h, 1, 4 and 14 weeks after exposure	Transient signs of fibrosis
Park E-J et al., **2009**	12 nm SAS particles from Degussa	ICR mice	Intratracheal instillation	Single dose	SiO_2 NPs increased the distribution of cytotoxic T cell, NK cell, and NKT cell, and induced subchronic inflammatory response
Other route					
Park E-J et al. **2011**	12 nm SAS particles from Degussa	ICR mice	Intraperitoneal injection	Single dose: 50 mg/kg Sampling at 3 days post exposure	ROS and pro-inflammatory responses
Cho MJ et al., **2009**	SiNPs from BITERIALS Co. Ltd. Korea	BALB/c mouse	Intravenous	Single dose: 50 mg/kg 12, 24, 48, and 72h, 7 days following injection	Incidence and severity of inflammatory response was transiently increased with injection of 200 and 100 nm SiNPs within 12 h. But there was no significant response related to injection of 50 nm particles. The SiNPs of 50, 100 and 200 nm were cleared via urine and bile. SiNPs were trapped by macrophages in the spleen and liver and remained there until 4 weeks after the single injection.
REVIEW	**Merget, R. et al., 2002: Health hazards due to the inhalation of amorphous silica**				
Gärtner, H, **1952** Klosterkötter, W,	Aerosil R972, Aerosil 200, R	Rabbits, Rats, Guinea			It was concluded that "there was no evidence for a fibrogenic effect of intentionally manufactured SAS to the human lung. Animal

1953 Schepers, GWH, Delahant, AB, **1957** Schepers, GWH, Durkan, TM, **1957** Schepers, GWH, Durkan, TM, **1957** Schepers, GWH, **1959** Schepers, GWH, **1962** Klosterkötter, W, **1965** Schepers, GWH, **1981** Groth, DH, **1981** Reuzel, PG, **1991** Lee, KP, **1993** Lewinson, J, **1994** Warheit, D, **1995**	974, Aerosil n.s., Hi-Sil 233, Sipernat 22S, Zeofree 80, Ludox, S.gel and precipitated n.s., fumed n.s., pyrogenic n.s.	Pigs, Monkeys			studies showed no persistent silicotic nodules even in long term inhalation experiments with high concentrations of SAS that are probably not encountered in workplace. This contrasts with inhalation experiments using crystalline silica which clearly demonstrated such effects. Although some collagen formation has been described in animals exposed to SAS, this is at least partially reversible after discontinuation of exposure. However, some studies described a minor persistent interstitial collagen deposition. Bronchitis, airway obstruction and emphysema were considered by few studies as outcome variables. Such effects in workers exposed to SAS have been described, but the importance of confounders cannot be quantified sufficiently in these studies. Inflammatory responses and emphysema have been described in a number of animal studies, especially in rats and monkeys. Thus, parameters assessing bronchitis, airway obstruction and emphysema had to be considered in further epidemiological studies as primary outcome variable."

**** it was not possible to verify what type of silicon dioxide was used from the information in the article, and consulting the web page of the listed company did not produce any information either.***

Table 17. Overview of study summaries provided for Acute Toxicity

Material (year of study)	Test Organism / System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings	Contributor	Comment
Inhalation							
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1983)**	Rat (Wistar, male and female)	Acute Inhalation Toxicity (OECD TG 403)	4 h maximum attainable concentration: 691 mg/m^3 (range: 650 - 725 mg/m3) Nominal concentration: 36.7 g/m^3	LC0 ≥ 0.69 mg/L air (analytical) LD50 ≥ 0.69 mg/L air (analytical)	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.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1983	Air exchange of the inhalation chamber was lower than recommended 0.8/h instead of 10 - 15/h.
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1983)**	Rat (Wistar, male and female)	Acute Inhalation Toxicity (OECD TG 403)	4 h maximum attainable concentration: 691 mg/m^3 (range: 650 - 725 mg/m3) Nominal concentration: 36.7 g/m^3	LD50 ≥ 0.69 mg/L air(analytical)	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.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1983	Air exchange of the inhalation chamber was lower than recommended 0.8/h instead of 10 - 15/h.
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1983)**	Rat (Wistar, male and female)	Acute Inhalation Toxicity (OECD TG 403)	4 h maximum technically attainable analytical concentration: av. 139 mg/m^3 (range 110 - 190 mg/m^3) Nominal concentration: 16.7 g/m^3	LC0 ≥ 0.14 mg/L air LD50 ≥ 0.14 mg/L air (analytical)	Restlessness, half-closed eyes Slight decrease or stagnation on day 2, but not related to previous exposure (note: by mistake animals were deprived of water for 16 h directly after exposure.) No clinical symptoms and no findings at autopsy after 14 d post-treatment.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1983	Air exchange of the inhalation chamber was lower than recommended 0.8/h instead of 10 - 15/h.

Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1981)**	Rat (Sprague-Dawley male and female)	Acute Inhalation Toxicity (OECD TG 403)	4 h Analytical concentration: 2.08 mg/L (average of 10 samples with a range from 1.63 to 2.70 mg/L, one outlier with 0.45 mg/L) Nominal concentration: 58.8 mg/L	LC0 ≥ 2.08 mg/L air (analytical) LD50 > 2.08 mg/L air (analytical) LC0 > 58.8 mg/L air (nominal) LD50 > 58.8 mg/L air (nominal)	No animals died. Nasal discharge during exposure, crusty eyes, crusty nose and alopecia at days post-exposure. No macroscopic organ lesions, but in one animal discoloration of the lung.	Data provided by BIAC. Tests performed by Toxigenics Inc., (USA) in 1981	The highest attainable exposure concentration was limited for technical reasons
Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(1983)**	Rat (Wistar, male and female)	Acute Inhalation Toxicity (OECD TG 403)	Maximum technically attainable analytical concentration: av. 139 mg/m^3 (range 110 - 190 mg/m^3) Nominal concentration: 16.7 g/m^3	LC0 ≥ 0.14 mg/L air LD50 ≥ 0.14 mg/L air (analytical	Restlessness, half-closed eyes Slight decrease or stagnation on day 2, but not related to previous exposure (note: by mistake animals were deprived of water for 16 h directly after exposure.) No clinical symptoms and no findings at autopsy after 14 d post-treatment.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1983	
Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(1981)**	Rat (Sprague-Dawley male and female)	Acute Inhalation Toxicity (OECD TG 403)	4 h Analytical concentration: 2.08 mg/L Nominal concentration: 58.8 mg/L	LC0 ≥ 2.08 mg/L air (analytical) LD50 > 2.08 mg/L air (analytical) LC0 > 58.8 mg/L air (nominal) LD50 > 58.8 mg/L air (nominal)	No animals died. Nasal discharge during exposure, crusty eyes, crusty nose and alopecia at days post-exposure. No macroscopic organ lesions, but in one animal discoloration of the lung.	Data provided by BIAC. Tests performed by Toxigenics Inc., (USA) in 1981	The highest attainable exposure concentration was limited for technical reasons
Oral							
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN	Rat (Sprague-Dawley, male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 1000, 2500, and 5000 mg/kg bw (pre-study); 5000 mg/kg bw (main study)	LD50 > 5000 mg/kg bw	No signs of toxicity	Data provided by BIAC. Tests performed by IFT (F) in 1986	

(1986)							
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1977)**	Rat (Sprague-Dawley, male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 2000 and 5000 mg/kg bw	LD50 > 5000 mg/kg bw	No signs of toxicity	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie (LPT) (GER) in 1977	
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1986)**	Rat (Sprague-Dawley male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 1000, 2500, and 5000 mg/kg bw (pre-study); 5000 mg/kg bw (main study)	LD50 > 5000 mg/kg bw	No signs of toxicity	Data provided by BIAC. Tests performed by IFT (F) in 1986	
Substance declared equivalent to **NM -201** (precipitated) Associated characterisation data not available to the WPMN **(1977)**	Rat (Sprague-Dawley male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 2000 and 5000 mg/kg bw	LD50 > 5000 mg/kg bw	No signs of toxicity	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie (LPT) (GER) in 1977	
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1977)**	Rat (Sprague-Dawley male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 2000 and 3300 mg/kg bw.	LD50 > 3300 mg/kg bw	No signs of toxicity. Body weight: slight reduction of 4 - 8 %, measured at days 1, 2, and 14 Feed consumption reduced in the 2000 mg group (10 , 4, 6 % at day 1, 2 and 14)	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie (LPT) (GER) in 1977	
Substance declared equivalent to **NM-202** (pyrogenic) Associated	Mouse (Swiss male)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 178, 316, 562, 1000, 1780 and 3160 mg/kg The test substance was given	LD50 > 3160 mg/kg bw	No adverse signs of toxicity in any animal during the study, no macroscopic lesions upon necropsy after 14-d	Data provided by BIAC. Tests performed by Hazelton Laboratories, (USA) in 1964	

characterisation data not available to the WPMN **(1964)**			by gavage at variable volumes, at maximum 10 ml/kg.		observation.		
Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(1977)**	Rat (Sprague-Dawley male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 2000 and 3300 mg/kg bw.	LD50 > 3300 mg/kg bw	Slight reduction of body weight of 4 - 8 %, measured at days 1, 2, and 14 Feed consumption reduced in the 2000 mg group (10, 4, 6 % at day 1,2 and 14)	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie (LPT) (GER) in 1977	
Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(1964)**	Mouse (Swiss male)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 178, 316, 562, 1000, 1780 and 3160 mg/kg The test substance was given by gavage at variable volumes, at maximum 10 ml/kg.	LD50 > 3160 mg/kg bw	No adverse signs of toxicity in any animal during the study, no macroscopic lesions upon necropsy after 14-d observation	Data provided by BIAC. Tests performed by Hazelton Laboratories, (USA) in 1964	
Substance declared equivalent to **NM-200, NM-201, NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1990)**	Rat (Wistar, male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) Single administration 5110 mg/kg bw 237 mg/mL	LD50 > 5000 mg/kg bw	No signs of toxicity	Data provided by BIAC. Tests performed by ASTA Pharma AG in 1990	
Substance declared equivalent to **NM-200, NM-201, NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1978)**	Rat (Sprague-Dawley male and female)	Acute Oral Toxicity (OECD TG 401)	Oral (gavage) 10000, 12600, 15800, and 20000 mg/kg	LC0 > 2000 mg/kg bw	no clinical symptoms; after 1 day the stools were white coloured (reversible after 2 days)	Data provided by BIAC. Tests performed by Huntingdon Research Center (HRC) in 1978	

Dermal							
Substance declared equivalent to **NM-200, NM-201, NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1978)**	Rabbit (New Zealand White)	Standard acute method under occlusive conditions	24 h 2000, 3000, 4000, and 5000 mg/kg	LD50 > 5000 mg/kg bw	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.	Data provided by BIAC. Tests performed by Huntingdon Research Center (HRC) in 1978	

5.3 Irritation

Table 18 provides an overview of study summaries provided for skin and eys irritation studies. The data is generated before the WPMN set up its testing programme, and it is stated that the material tested is equivalent to the NM-series.

Table 18. Overview of study summaries provided for Skin and Eye Irritation.

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Main findings**	**Contributor**
Skin irritation					
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1992)**	Rabbit (New Zealand White)	National standard protocol (No. IPC/05-92) corresponding to US EPA	24 h 190 mg/0.5 mL Observation period: 3 days intact and abraded skin	Slight erythemas were seen in 4/6 animals 0.5 h after 24 h exposure. No signs of irritation after 72 h.	Data provided by BIAC. Tests performed by Hazelton (F) in 1992
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation	Rabbit (New Zealand White)	Acute Dermal Irritation / Corrosion (OECD TG 404) Patch-Test; Hazardous Substances, Part 191, Section	24 h 0.5 g Observation period: 14 days	No signs of irritation	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und

data not available to the WPMN **(1978)**		11, FDA, Washington, 1965	6 (intact skin) 6 (abraded skin)		Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1978)**	Rabbit (New Zealand White)	Acute Dermal Irritation / Corrosion (OECD TG 404) Patch-Test; Hazardous Substances, Part 191, Section 11, FDA, Washington, 1965	24 h 0.5 g Observation period: 14 days 6 (intact skin) 6 (abraded skin)	No signs of irritation	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1978)**	Rabbit (New Zealand White)	Acute Dermal Irritation / Corrosion (OECD TG 404) Patch-Test; Hazardous Substances, Part 191, Section 11, FDA, Washington, 1965	24 h 0.5 g Observation period: 14 days 6 (intact skin) 6 (abraded skin	No signs of irritation	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(1978)**	Rabbit (New Zealand White)	Acute Dermal Irritation / Corrosion (OECD TG 404) Patch-Test; Hazardous Substances, Part 191, Section 11, FDA, Washington, 1965	24 h 0.5 g Observation period: 14 days 6 (intact skin) 6 (abraded skin) (Observation period: 3 days)	No signs of irritation	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-200, NM-201, NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1991)**	Rabbit (White Russian)	Acute Dermal Irritation / Corrosion (OECD TG 404)	24 h 0.5 g Observation period: 14 days	The single application (4 hours, occlusive patch) of 0.5 g 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	Data provided by BIAC. Tests performed by ASTA Pharma AG in 1991

				system	
Substance declared equivalent to **NM-200, NM-201, NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1992)**	Rabbit (New Zealand White)	National standard protocol (No. IPC/05-92) corresponding to US EPA	24 h 190 mg Observation period: 3 days intact and abraded skin	Slight erythemas were seen in 4/6 animals 0.5 h after 24 h exposure. No signs of irritation after 72 h.	Data provided by BIAC. Tests performed by Hazelton (F) in 1992
Eye irritation					
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1978)**	Rabbit (New Zealand White)	Draize-Test; Hazardous Substances, FDA	24 h (exposure) 100 mg Observation period: 96 h	No irritating response at any time after exposure (24 - 96 h).	Data provided by BIAC Tests performed by Laboratorium für Pharmakologie und Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-201** and **NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1978)**	Rabbit (New Zealand White)	Draize-Test; Hazardous Substances, FDA	24 h 100 mg Observation period: 96 h	No irritating response at any time after exposure (24 - 96 h)	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1978)**	Rabbit (New Zealand White)	Draize-Test; Hazardous Substances, FDA	24 h 100 mg Observation period: 96 h	No irritating response at any time after exposure (24 - 96 h	Data provided by BIAC. Tests performed by Laboratorium für Pharmakologie und Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-203**	Rabbit (New Zealand	Draize-Test; Hazardous Substances, FDA	24 h	No irritating response at any time after exposure (24 - 96 h)	Data provided by BIAC. Tests performed by

(pyrogenic) Associated characterisation data not available to the WPMN **(1978)**	White)		100 mg Observation period: 96 h		Laboratorium für Pharmakologie und Toxikologie LPT (GER) in 1978
Substance declared equivalent to **NM-200, NM-201, NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1991)**	Rabbit (White Russian)	Acute Eye Irritation / Corrosion (OECD TG 405)	24 h 100 mg Observation period: 7 days	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).	Data provided by BIAC. Tests performed by ASTA Pharma AG in 1991

5.4 Sensitisation

The OECD WPMN did not list this end-point for investigation and thus no testing under the WPMN was undertaken.

5.5 Repeated dose toxicity

The tables below summarises the available information. Table 19 summarises the information from scientific literature available for repeated dose toxicity; the exposure is intratracheal instillation (1 study) and oral exposure (1 study).

Table 19. Repeated dose toxicity. Summary of information from scientific literature.

Reference	Material/ Size	Test Organism / System	Method	Exposure/ dose	Main findings
Ernst et al. **2002**	Aerosil 150 (fumed SiO_2), primary particles 14 nm, surface	Wistar WU female rats	Intratracheal instillation	2 groups: 20 and 30 instillations at intervals of 2 weeks	After repeated Intratracheal instillation exposure, elevation of biomarkers of cytotoxicity in BAL fluids were observed, but it did not affect the ability of the lung cells to respond to LPS (>< quartz and carbon black). This can be attributed to the rapid elimination of amorphous SiO_2 from lungs that was detected in a separate

	specific area 300 m^2/g			0.5 mg	experiment mentioned in the article. Following intratracheal instillation, about 85% of the SAS was removed within 2 days, followed by a half-time of 11 days. It was concluded for the carcinogenicity study that chronic inflammation without persistent dust particles could be induced by 20 instillations at intervals of two weeks with low single doses of 0.5 mg. Intratracheal instillation of SiO_2 produced marked inflammatory lesions in the lungs of all treatment groups. The inflammation caused by amorphous SiO_2 was characterized by a lack of alveolar lipoproteinosis and relatively low numbers of intra-alveolar macrophages. The majority of the macrophages were foamy but not necrotizing. Amorphous SiO_2 also produced a pronounced but very localized interstitial fibrosis (interstitial fibrotic granulomas). These are believed to develop from acute alveolitis observed after a single administration of amorphous SiO_2.
So SJ et al., **2008**	Nano-: 30 nm Micro-: 30 µm obtained by rice husk	BALB/c mouse C57BL/6J mouse	Oral route (food)	10 weeks 1 % SiO_2 in diet	Nano-sized Si particles had a toxic effect on the liver even though there was no significant different on the health in total fed amount of 140 g Si/kg mouse.

Table 20 summarises the data for short term (repeated) dose toxicity for the following exposure routes: Oral, IV route and Intratracheal instillation (For these three routes the data is from Nanogenotox and the test material from the NM-series). Furthermore, Inhalation exposure data was provided by BIAC and the data is generated before the WPMN set up its testing programme, and it is stated that the material tested is equivalent to the NM-series.

Results from sub-acute toxicity studies are reported in

Table 21 (oral gavage exposure) and from sub-chronic toxicity studies in Table 22 (inhalation exposure) where the data is provided by BIAC and the data is generated before the WPMN set up its testing programme, and it is stated that the material tested is equivalent to the NM-series.

Outcomes for chronic toxicity study (inhalation route) are reported in Table 23, the data is generated before the WPMN set up its testing programme, and it is stated that the material tested is equivalent to the NM-series.

Table 20. Overview of study summaries provided for Repeated Dose Toxicity (short-term)

Material (year of study)	**Test Organism/System**	**Method**	**Exposure/ dose**	**Derived effect value (dose descriptor)**	**Main findings**	**Contributor**	**Comment**
Oral							
NM-200 (precipitated) **(2013)**	Rat (Sprague-Dawley. male and female)	Oral route (on 5 consecutive days, day 1-5)	6.0 mg/ml was prepared in sterile normal saline (NaCl 0.9% w/v). Groups received daily a dose of 20 mg/kg bw. The repeated dose groups received a total cumulative dose of 100 mg/kg bw after 5 days treatment.		Tissue deposition following repeated oral administration to a total dose of 100 mg SAS / kg bw was negligible or absent. Slight differences in gender/material investigated are apparent, though the limited absorption makes it difficult to draw any conclusions. In terms of effects, at sacrifice at day 6, altered organ weight (e.g. liver, lungs, uterus) was observed. Preliminary histopathological examinations; increased ratio between white and red pulp of the spleen and altered vascularisation in NM-200 treated female group.	Data provided by Nanogenotox performed by ISS (I) in 2013	
NM-203 (pyrogenic) **(2013)**	Rat (Sprague-Dawley. male and female)	Oral route (on 5 consecutive days, day 1-5)	6.0 mg/ml was prepared in sterile normal saline (NaCl 0.9% w/v). Groups received daily a dose of 20 mg/kg bw. The repeated dose groups received a total cumulative dose of 100 mg/kg bw after 5 days treatment.		Tissue deposition following repeated oral administration to a total dose of 100 mg SAS /kg bw was negligible or absent. Slight differences in gender/material investigated are apparent, though the limited absorption makes it difficult to draw any conclusions. In terms of effects, at sacrifice at day 6, altered organ weight (e.g. liver, lungs, uterus) was observed. Preliminary histopathological examinations showed increased ratio between white and red pulp of the spleen and apoptosis in NM-203 treated male and female groups; increased ratio between white and red pulp of the spleen and altered vascularisation in liver in NM-203 treated female group.	Data provided by Nanogenotox performed by ISS (I) in 2013	
IV route							
NM-200	Rat (Sprague-	IV route	6.0 mg/ml was prepared in sterile normal saline		After repeated dose IV administration, Si peaked in blood at 20 min in females and at 30 min in males. At day 6, Si	Data provided by	

(precipitated) **(2013)**	Dawley. male and female)	(on 5 consecutive days, day 1-5)	(NaCl 0.9% w/v). Groups received daily a dose of 20 mg/kg bw. The repeated dose groups received a total cumulative dose of 100 mg/kg bw after 5 days treatment.		concentrations were very high in liver, spleen and lungs, with marked particle and gender-related differences, and above the LOQ in other organs as well. At days 14 and 30 considerable amounts of Si were still present in liver, spleen and lungs of male rats. In female rats, the highest concentra-tion was present in the liver both at day 6 and day 90. In male rats the highest concentration was in the liver. At day 90 Si concentration in liver and spleen tissues of males and females were still distinctly higher than those in controls. Gross observation at sacrifice at day 90 showed specific effects on liver and spleen. Furthermore after repeated IV exposure, NM-200 treated male animals showed a slight reduction in weight gain compared to control animals.	Nanogenotox performed by ISS (I) in 2013 IT)	
NM-203 (pyrogenic) **(2013)**	Rat (Sprague-Dawley, male and female)	IV route (on 5 consecutive days, day 1-5)	6.0 mg/ml was prepared in sterile normal saline (NaCl 0.9% w/v). Groups received daily a dose of 20 mg/kg bw. The repeated dose groups received a total cumulative dose of 100 mg/kg bw after 5 days treatment.		After repeated dose IV administration, Si peaked in blood at 20 min in females and at 30 min in males. At day 6, Si concentrations were very high in liver, spleen and lungs, with marked particle and gender-related differences, and above the LOQ in other organs as well. At days 14 and 30 considerable amounts of Si were still present in liver, spleen and lungs of male rats. In female rats, similar concentrations were observed in liver and spleen both at day 6 and day 90 for NM-203. In male rats NM-203 showed the highest concentration in the spleen. For NM-203 male rats a much higher distribution was noted compared to female rats at day 6 after the repeated administrations. At day 90 Si concentration in liver and spleen tissues of males and females were still distinctly higher than those in controls, suggesting a longer time required for complete elimination of administered NMs from the body. Following both single and repeated dose administration, gross observation at sacrifice at day 90 showed specific effects on liver and spleen.	Data provided by Nanogenotox performed by ISS (I) in 2013	Only 5 exposure days; histopathology and organotoxicology limited; no clinical chemistry or haematology. Lung lavage cytology and biochemistry were used instead

Intratracheal instillation							
NM-200 (precipitated) **(2013)**	Rat (Sprague-Dawley, male)	Intratracheal instillation	1 administration at 0, 24 and 45 h 1.15; 2.3; 4.6 mg/kg bw/d		Dose-dependent increase in the number of neutrophils in BAL	Data provided by Nanogenotox performed INRS (F) in 2013	
Inhalation							
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(2003)**	Rat (Wistar, male and female)	Inhalation Toxicity: Method: in accordance with OECD TG 412 (1981) and directive 92/69/EEC, 29 Dec. 1992, but focus on the respiratory tract (lung and lymph nodes).	6 h/day for 5 days 1.16 (± 0.36), 5.39 (± 0.58), 25.2 (± 1.5) mg/m^3 Post-exposure period: 1 or 3 months	NOEC (acute/sub-acute) is at 1.16 mg/m3. The NOAEC could be defined as 5.39 mg/m^3	There are slight increases in lung weight of the high dose group, statistically significant absolute weights in male and relative in females and an increase in relative weights of tracheobronchial lymph nodes in females of the high dose group. After 5 days, the absolute numbers of neutrophils increase significantly in the high dose groups of both genders. The relative number of macrophages decreased concomitantly. In the mid dose group, neutrophils slightly increased. After recovery of one month, the cell stimulating effect passed away and were also absent after 3 months recovery for females, but noted in males without changes. Slights trends were also seen in the mid dose group, but only reflected on the relative neutrophil increase. Significant increases in enzymes and proteins were found only at the high dose exposure, which completely reverse after recovery. Hypertrophy and hyperplasia of the bronchiolar epithelium (high dose) were noticed in 1/5 males and in 2/5 females. Because of the very rare occurrence in the rats of that age, this lesion was considered treatment relative. A very slight to slight polymorphonuclear leukocytes infiltration (inflammation response) was noted at all dose levels. The incidence and the severity was not clearly	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 2003	Only 5 exposure days; histopathology and organotoxicology limited; no clinical chemistry or haematology. Lung lavage cytology and biochemistry were used instead

					dose-related, 1/5 very slight case at the low dose level in the male and in the female group respectively. This effect was occasionally observed in the recovery group (as well in the recovery control group). In recovery of the high dose group, a tendency of accumulation of alveolar macrophages and hyperemic capillary unusual type II hyperplasia in 1/5 males were noted.		
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(2003)**	Rat (Wistar male and female)	Inhalation Toxicity: Method: in accordance with OECD TG 412 (1981) and directive 92/69/EEC, 29 Dec. 1992, but focus on the respiratory tract (lung and lymph nodes).	6 h/day for 5 days 1.16 (± 0.36), 5.39 (± 0.58), 25.2 (± 1.5) mg/m^3 Post-exposure period: 1 or 3 months	NOEC (acute/sub-acute) is at 1.16 mg/m3. The NOAEC could be defined as 5.39 mg/m^3	There are slight increases in lung weight of the high dose group, statistically significant absolute weights in males and relative in females and an increase in relative weights of tracheobronchial lymph nodes in females of the high dose group. After 5 days, the absolute numbers of neutrophils increase significantly in the high dose groups of both genders. The relative number of macrophages decreased concomitantly. In the mid dose group, neutrophils slightly increased. After recovery of one month, the cell stimulating effect passed away and were also absent after 3 months recovery on females, but noted in males without changes. Slights trends were also seen in the mid dose group, but only reflected on the relative neutrophil increase. Significant increases in enzymes and proteins were found only at the high dose exposure, which completely reverse after recovery. Hypertrophy and hyperplasia of the bronchiolar epithelium (high dose) were noticed in 1/5 males and in 2/5 females. Because of the very rare occurrence in the rats of that age, this lesion was considered treatment relative. A very slight to slight polymorphonuclear leukocytes infiltration (inflammation response) was noted at all dose levels. The incidence and the severity was not clearly dose-related, 1/5 very slight case at the low dose level in the male and in the female group respectively. This effect was occasionally observed in the recovery group (as well	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 2003	Only 5 exposure days; histopathology and organotoxicology limited; no clinical chemistry or haematology. Lung lavage cytology and biochemistry were used instead

					in the recovery control group). In recovery of the high dose group, a tendency of accumulation of alveolar macrophages and hyperemic capillary unusual type II hyperplasia in 1/5 males were noted.		
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(2003)**	Rat (Wistar male)	Inhalation Toxicity: Method: in accordance with OECD TG 412 (1981) and directive 92/69/EEC, 29 Dec. 1992, but focus on the respiratory tract (lung and lymph nodes) Test substance was examined only in males because they had proven to be more sensitive than females, as observed in the first	6 h/day for 5 days 1.39 (± 0.15), 5.41 (± 0,34), 25.3 (0.9) mg/m^3 Post-exposure period: 1 or 3 months	LOAEC 5.41 mg/m^3 air (analytical) NOEC 1.39 mg/m^3 air (analytical)	Significant mean increases in relative and absolute lung weights of the mid- and high-dose groups. No increases in weight of the tracheobronchial lymph nodes were noticed. Very slight hypertrophy of the bronchiolar epithelium in 3/5 animal (mid-dose) and slight hypertrophy in 4/5 (high dose) were observed. No case occurred in the recovery group. Accumulation of alveolar macrophages accompanied by a few granulocytes / neutrophils in 3/5 animal (mid dose) and 5/5 (high dose) were noted. In 3/5 high dose animals, alveolar accumulation of macrophages was accompanied by an infiltration of polymorphonuclear leukocytes. Following recovery of one month, very slight macrophage accumulation was still present in the lungs in 3/5 high-dose animals, but without epithelial changes and leukocytes infiltrations. At that time, lymph nodes also contained aggregates of macrophages (1/5 mid-dose), 5/5 high dose). Following recovery of 3 months, local accumulation of macrophages was still present in the lungs of 2/5 high-dose animals. The lymph nodes of 1/5 mid-dose and 5/5 high dose animals still contained macrophages aggregates. After 5 days, absolute and relative number of neutrophils increased significantly in both the mid- and the high-dose groups, the relative number of macrophage decreases concomitantly. After 1 month recovery, the cell stimulating effects passed away, there were still slight but statistically significant increase of the percentages of the neutrophils counts with concomitant decrease in relative macrophage units but no longer after 3 months. Significant increases in enzymes, proteins and the TNF –	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 2003	Only 5 exposure days; histopathology and organotoxicology limited; no clinical chemistry or haematology. Lung lavage cytology and biochemistry were used instead

		study.			α levels were found at the mid- and high dose exposure.		
Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(2003)**	Rat (Wistar male))	Inhalation Toxicity: Method: in accordance with OECD TG 412 (1981) and directive 92/69/EEC, 29 Dec. 1992, but focus on the respiratory tract (lung and lymph nodes). Test substance was examined only in males because they had proven to be more sensitive than females, as observed in the first study.	6 h/day for 5 days 1.39 (± 0.15), 5.41 (± 0,34), 25.3 (0.9) mg/m^3 Post-exposure period: 1 or 3 months	LOAEC 5.41 mg/m^3 air (analytical) NOEC 1.39 mg/m^3 air (analytical)	Significant mean increases in relative and absolute lung weights of the mid- and high-dose groups. No increases in weight of the tracheobronchial lymph nodes were noticed. Very slight hypertrophy of the bronchiolar epithelium in 3/5 animal (mid-dose) and slight hypertrophy in 4/5 (high dose) were observed. No case occurred in the recovery group. Accumulation of alveolar macrophages accompanied by a few granulocytes / neutrophils in 3/5 animal (mid dose) and 5/5 (high dose) were noted. In 3/5 high dose animals, alveolar accumulation of macrophages was accompanied by an infiltration of polymorphonuclear leukocytes. Following recovery of one month, very slight macrophage accumulation was still present in the lungs in 3/5 high-dose animals, but without epithelial changes and leukocytes infiltrations. At that time, lymph nodes also contained aggregates of macrophages (1/5 mid-dose), 5/5 high dose). Following recovery of 3 months, local accumulation of macrophages was still present in the lungs of 2/5 high-dose animals. The lymph nodes of 1/5 mid-dose and 5/5 high dose animals still contained macrophages aggregates. After 5 days, absolute and relative number of neutrophils increased significantly in both the mid- and the high-dose groups, the relative number of macrophage decreases concomitantly. After 1 month recovery, the cell stimulating effects passed away, there were still slight but statistically significant increase of the percentages of the neutrophils counts with concomitant decrease in relative macrophage units but no longer after 3 months. Significant increases in enzymes, proteins and the TNF – α levels were found at the mid- and high dose exposure.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 2003	Only 5 exposure days; histopathology and organotoxicology limited; no clinical chemistry or haematology. Lung lavage cytology and biochemistry were used instead
Substance	Rat (Wistar	Inhalation	6 h/day for 5 days	NOEC mean	There are slight increases in lung weight of the high dose	Data provided	Only 5

declared equivalent to **NM-204** (precipitated) Associated characterisation data not available to the WPMN **(2003)**	male and female)	Toxicity: Method: in accordance with OECD TG 412 (1981) and directive 92/69/EEC, 29 Dec. 1992, but focus on the respiratory tract (lung and lymph nodes).	1.16 (± 0.36), 5.39 (± 0.58), 25.2 (± 1.5) mg/m^3 Post-exposure period: 1 or 3 months	1.16 mg/m³ air (analytical) (Histopathology: based on the absence of substance-related effects, in particular absence of a pulmonary response (inflammation reaction) NOAEC mean 5.39 mg/m³ air (analytical)(Histopathology: based on the pulmonary response (inflammation reaction)) LOAEC mean 25.2 mg/m³ air (analytical)	group, statistically significant absolute weights in male and relative in females and an increase in relative weights of tracheobronchial lymph nodes in females of the high dose group. After 5 days, the absolute numbers of neutrophils increase significantly in the high dose groups of both genders. The relative number of macrophages decreased concomitantly. In the mid dose group, neutrophils slightly increased. After recovery of one month, the cell stimulating effect passed away and were also absent after 3 months recovery for females, but noted in males without changes. Slights trends were also seen in the mid dose group, but only reflected on the relative neutrophil increase. Significant increases in enzymes and proteins were found only at the high dose exposure, which completely reverse after recovery. Hypertrophy and hyperplasia of the bronchiolar epithelium (high dose) were noticed in 1/5 males and in 2/5 females. Because of the very rare occurrence in the rats of that age, this lesion was considered treatment relative. A very slight to slight polymorphonuclear leukocytes infiltration (inflammation response) was noted at all dose levels. The incidence and the severity was not clearly dose-related, 1/5 very slight case at the low dose level in the male and in the female group respectively. This effect was occasionally observed in the recovery group (as well in the recovery control group). In recovery of the high dose group, a tendency of accumulation of alveolar macrophages and hyperemic capillary unusual type II hyperplasia in 1/5 males were noted.	by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 2003	exposure days; histopathology and organotoxicology limited; no clinical chemistry or haematology. Lung lavage cytology and biochemistry were used instead

Table 21. Overview of study summaries provided for Repeated Dose Toxicity (subacute) *(There are not comments thus that column is not included)*

Material (Year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Derived effect value (dose descriptor)**	**Main findings**	**Contributor**
NM-200 (precipitated) **(2011)**	Rat (Wistar, male)	Oral (gavage) OECD TG 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	1/day for 28 days 100, 300, 1000 mg/kg bw/d Rats were treated daily either with the vehicle (0.5% methylhydroxypropylcellulose, control groups) or with Synthetic Amorphous Silica suspended in the vehicle for 28 consecutive days.	NOEL = 1000 mg/kg bw/d (actual dose received)	No death occurred during the study and no adverse clinical symptoms were observed. No effects on food consumption or body weight were seen. The measurements of the spontaneous locomotor activity and the functional observational battery displayed no influence by the treatment. Evaluation of haematological and clinical chemistry parameters did not reveal any treatment related effects. Decreases of the partial thromboplastin time (PTT), white blood cell count (WBC) and lymphocyte count (LYMC) as well as cholinesterase (CHE) and glucose (GLUC) in group 3 (mid dose) after 28 d of exposure were considered not treatment-related. Creatinin kinase (CK) and blood urea (UREA) concentration were mildly decreased in group 6 (high dose recovery) after a two week recovery period. During necropsy, no substance-related findings were observed. No effects were seen on organ weights or the organ weight to bodyweight ratio. During histopathological examination, no substance-related findings were observed in the examined organs of males of the control and high dose group. Toxicological analysis of silica ion concentration (non-GLP) in blood, kidney and liver tissue did not reveal differences between the control and the high dose group. This result is most likely due to the naturally occurring high background values of silica. Transmission electron microscopy (non-GLP) found electron dense structures composed of irregular homogenous to fine granular material in the cytoplasm of mesenteric lymph nodes cells, liver cells and kidney cells of all animals from the control and from the high dose group. The granular structures measured only few nanometers. However, these structures did not have the shape or appearance of amorphous material such as synthetic amorphous silica, (SAS) NM-200.	Data provided by BIAC. Tests performed by Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) (GER) in 2011

Table 22. Overview of study summaries provided for Repeated Dose Toxicity (subchronic)

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Derived effect value (dose descriptor)**	**Main findings**	**Contributor**	**Comment**
Inhalation							
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar, male and female)	Subchronic Inhalation Toxicity: 90-Day (OECD TG 413)	90 days 6 hours/day, 5 days/week 35 mg/m^3 (mean analytical values) Basis analytical conc. 30 mg/m^3 (target concentration	No NOAEC identified (Test substance at a level of 30 mg/m^3 induced generally mild changes, which quickly recovered during the exposure period.)	A slight decrease of body weight (- 5 %) by 13 weeks exposure was observed (still at – 4 % after 52 weeks post exposure). No significant effects in haematology were detected but white blood cells count elevated in both male and female groups at the end of exposure period, but it was not clear-ly attributable to the increase in the number of neutrophilic leukocytes. After 13 weeks recovery, neutrophil count still tended to be higher in males and females, and normalised by 28 weeks of recovery. No changes in heart, thyroids, adrenals, testes, brain, spleen and kidneys weights were observed, but the relative mean of lungs weighs slightly increased (≈ x 1.3). Thymus weight increased as well. Swollen lungs and enlarged mediastinal lymph nodes were noted. The effects gradually subsided after the exposure period. Lung weights were normalised after 13 weeks recovery in males and females. In the lung, accumulation of alveolar macrophage, intra-alveolar polymorphonuclear leukocytes and increased septal cellularity in males and females were noted. Treatment related microscopic changes in the nasal region were found at the end of the exposure, such as very slight local necrosis and slight atrophy of the olfactory epithelium, intracystoplasmic proteinaeceous droplets. Accumulation of macrophages was seen in the mediastinal lymph nodes (disappearing after 39 week post exposure). Collagen content in the lungs slightly increased at the end of exposure. During the recovery period, all changes disappeared mostly within 13 - 26 weeks post exposure. Silica could be detected in lungs only in relatively small amounts at the end of the exposure periods. On average 0.5 mg per lung in male and 0.35 mg per lung in female groups, decreasing over time and no longer measurable after 39 weeks post exposure were found.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987	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

Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar, male and female)	Subchronic Inhalation Toxicity: 90-Day (OECD TG 413)	90 days 6 hours/day, 5 days/week 35 mg/m^3 (mean analytical values) Basis analytical conc. 30 mg/m^3 (target concentration	No NOAEC identified (Test substance at a level of 30 mg/m3 induced generally mild changes, which quickly recovered during the exposure period.)	A slight decrease of body weight (- 5 %) by 13 weeks exposure was observed (still at – 4 % after 52 weeks post exposure). No significant effects in haematology were detected but white blood cells count elevated in both male and female groups at the end of exposure period, but it was not clear-ly attributable to the increase in the number of neutrophilic leukocytes. After 13 weeks recovery, neutrophil count still tended to be higher in males and females, and normalised by 28 weeks of recovery. No changes in heart, thyroids, adrenals, testes, brain, spleen and kidneys weights were observed, but the relative mean of lungs weighs slightly increased (≈ x 1.3). Thymus weight increased as well. Swollen lungs and enlarged mediastinal lymph nodes were noted. The effects gradually subsided after the exposure period. Lung weights were normalised after 13 weeks recovery in males and females. In the lung, accumulation of alveolar macrophage, intra-alveolar polymorphonuclear leukocytes and increased septal cellularity in males and females were noted. Treatment related microscopic changes in the nasal region were found at the end of the exposure, such as very slight local necrosis and slight atrophy of the olfactory epithelium, intracystoplasmic proteinaeceous droplets. Accumulation of macrophages were seen in the mediastinal lymph nodes (disappeared after 39 week post exposure). Collagen content in the lungs slightly increased at the end of the exposure. During the recovery period, all changes disappeared mostly within 13 - 26 weeks post exposure. Silica could be detected in lungs only in relatively small amounts at the end of the exposure periods. On average 0.5 mg per lung in male animal group, 0.35 mg per lung of female groups, decreasing over time and no longer measurable after 39 weeks post exposure were found.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987	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
Substance declared equivalent to **NM-202** (pyrogenic) Associated	Rat (Wistar, male and female)	Subchronic Inhalation Toxicity: 90-Day (OECD TG 413)	90 days 6 hours/day, 5 days/week 1.3, 5.9 or 31 mg/m^3 (mean	NOAEC = 1.3 mg/m³ air (analytical) NOEC < 1.3 mg/m³	The respiration rate was increased (concentration related). No effect in female weights in aldose levels was detected. Depressive effects on males weight were found (1 mg/m^3 slightly at day 14 (- 5 %), 6 mg/m^3 slightly from day 49 to 77 (- 6 to -5 %), 30 mg/m^3 significant throughout exposure (-10 to -7 %)). No difference from control at day 45 observed. No haematology effects were found for 1 mg/m^3 group. For the 6 mg/m^3 group, white blood cell count elevated in males and females due to	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food	Special modifications as compared with standard study: Focus upon lung, respiratory tract,

characterisation data not available to the WPMN **(1987)**			analytical values) 1, 6 and 30 mg/m^3 (target concentrations)	air (analytical) LOAEC = 5.9 mg/m^3 air (analytical)	increase in the number of neutrophilic leukocytes but concentration response relation was poor. After 3 months recovery, these blood parameters were normalised. For 30 mg/m^3 group, red blood cells and haemoglobin were statistically higher in males, but not in females. White blood cells count elevated in males and females due to increase of the number of neutrophilic leukocytes at 3 months of recovery. In females, a slight increase above the control group still existed after 6 months of recovery. Swollen lungs and enlarged mediastinal lymph nodes at the end of recovery was found (treatment related degrees of severity). No lung weight effect was found for 1 mg/m^3 group, but an increase was observed for the 6 (1.7 x for males and 1.4 x for females)and 30 mg/m^3 (2.3 x for males and 2.0 x for females) groups. For the 6 and 30 mg/m^3 groups, collagen content in the lungs was clearly increased, most pronounced in males. The above mentioned effects gradually subsided after the exposure period. But in males exposed to 6 to 30 mg/m^3, the collagen content was still above control values at the end of the study. Granuloma-like lesions were seen in animals at the end of exposure period and after the 13 weeks of recovery. They did not show fibroblastic activity and hyanilisation and regressed during recovery. Accumulation of macrophages was seen in the mediational lymph nodes (disappeared week 39). Treatment related microscopic changes in the nasal region were occasionally found at the end of exposure period such as focal necrosis and slight atrophy of the olfactory epithelium. Interstitial fibrosis was not noted directly after the exposure period, but appeared with a delay. It was observed for the first time after 13 weeks post exposure, increasing incidence especially for 30 mg/m^3 and less for 6 mg/m^3. It decreased in severity and frequency until the end of the study. All types of pulmonary lesions were more marked in males than in females. The level of 1.3 mg/m^3 induced only slight changes after 13 weeks post exposure which generally recovered quickly. Morphological changes after 13 weeks exposure are considered statistically significant at 1.3 mg/m^3. Silica could be detected in lungs only in relatively small amounts at the end of exposure period. Only one male exposed to 30 mg/m^3 show a small amount of silica in the regional lymph nodes. 90 days after termination of exposure, no silica could be recovered from any animal.	Research, Zeist (NL) in 1987)	and lymph nodes. Post-exposure recovery period up to one year.

Substance declared equivalent to **NM-203** (pyrogenic) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar, male and female)	Subchronic Inhalation Toxicity: 90-Day (OECD TG 413)	90 days 6 hours/day, 5 days/week 1.3, 5.9 or 31 mg/m^3 (mean analytical values) 1, 6 and 30 mg/m^3 (target concentrations)	NOAEC = 1.3 mg/m³ air (analytical) NOEC < 1.3 mg/m³ air (analytical) LOAEC = 5.9 mg/m³ air (analytical)	The respiration rate was increased (concentration related). No effect in female weights in aldose levels was detected. Depressive effects on males weight were found (1 mg/m^3 slightly at day 14 (- 5 %), 6 mg/m^3 slightly from day 49 to 77 (- 6 to -5 %), 30 mg/m^3 significant throughout exposure (-10 to -7 %)). No difference from control at day 45 observed. No haematology effects were found for 1 mg/m^3 group. For the 6 mg/m^3 group, white blood cell count elevated in males and females due to in-crease in the number of neutrophilic leukocytes but concentration response relation was poor. After 3 month recovery, these blood parameters were normalised. For 30 mg/m^3 group, red blood cells and haemoglobin were statistically higher in males, but not in females. White blood cells count elevated in males and females due to increase of the number of neutrophilic leukocytes at 3 months of recovery. In females, a slight increase above the control group still existed after 6 months of recovery. Swollen lungs and enlarged mediastinal lymph nodes at the end of recovery was found (treatment related degrees of severity). No lung weight effect was found for 1 mg/m^3 group, but an increase was observed for the 6 (1.7 x for males and 1.4 x for females)and 30 mg/m^3 (2.3 x for males and 2.0 x for females) groups. For the 6 and 30 mg/m^3 groups, collagen content in the lungs was clearly increased, most pronounced in males. The above mentioned effects gradually subsided after the exposure period. But in males exposed to 6 to 30 mg/m^3, the collagen content was still above control values at the end of the study. Granuloma like lesions were seen in animals at the end of exposure period and after the 13 weeks of recovery. They did not show fibroblastic activity and hyanilisation and regressed during recovery. Accumulation of macrophages was seen in the mediational lymph nodes (disappeared week 39). Treatment related microscopic changes in the nasal region were occasionally found at the end of exposure period such as focal necrosis and slight atrophy of the olfactory epithelium. Interstitial fibrosis was not noted directly after the exposure period, but appeared with a delay. It was observed for the first time after 13 weeks post exposure, increasing incidence especially for 30 mg/m^3 and less for 6 mg/m^3. It decreased in severity and frequency until the end of the study. All types of pulmonary lesions were more marked in males than in females. The level of 1.3 mg/m^3 induced only slight changes	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987)	Special modifications as compared with standard study: Focus upon lung, respiratory tract, and lymph nodes. Post-exposure recovery period up to one year.

					after 13 weeks post exposure which generally recovered quickly. Morphological changes after 13 weeks exposure are considered statistically significant at 1.3 mg/m^3. Silica could be detected in lungs only in relatively small amounts at the end of exposure period. Only one male exposed to 30 mg/m^3 showed a small amount of silica in the regional lymph nodes. 90 days after termination of exposure, no silica could be recovered from any animal.		
Substance declared equivalent to **NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1987)**	Rat (Wistar, male and female)	Subchronic Inhalation Toxicity: 90-Day (OECD TG 413)	90 days 6 hours/day, 5 days/week 35 mg/m^3 (mean analytical values) Basis analytical conc. 30 mg/m^3 (target concentration	No NOAEC identified (Test substance at a level of 30 mg/m3 induced generally mild changes, which quickly recovered during the exposure period.)	A slight decrease of body weight (- 5 %) by 13 weeks exposure was observed (still at – 4 % after 52 weeks post exposure). No significant effects in haematology were detected but white blood cells count elevated in both male and female groups at the end of exposure period, but it was not clearly attributable to the increase in the number of neutrophilic leukocyte. After 13 weeks recovery, neutrophil count still tended to be higher in males and females, and normalised by 28 weeks of recovery. No changes in heart, thyroids, adrenals, testes, brain, spleen and kidneys weights were observed, but the relative mean of lungs weighs slightly increased (≈ x 1.3). Thymus weight increased as well. Swollen lungs and enlarged mediastinal lymph nodes were noted. The effects gradually subsided after the exposure period. Lung weights were normalised after 13 weeks recovery in males and females. In the lung, accumulation of alveolar macrophage, intra-alveolar polymorphonuclear leukocytes and increased septal cellularity in males and females were noted. Treatment related microscopic changes in the nasal region were found at the end of the exposure, such as very slight local necrosis and slight atrophy of the olfactory epithelium, intracystoplasmic proteinaeceous droplets. Accumulation of macrophages was seen in the mediastinal lymph nodes (disappeared after 39 week post exposure). Collagen content in the lungs slightly increased at the end of the exposure, During the recovery period, all changes disappeared mostly within 13 to 26 weeks post exposure. Silica could be detected in lungs only in relatively small amounts at the end of the exposure periods. On average 0.5 mg per lung in male animal group, 0.35 mg per lung of female groups, decreasing over time and no longer measurable after 39 weeks post exposure were found.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL) in 1987	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

Oral							
Substance declared equivalent to **NM-200** (precipitated) Associated characterisation data not available to the WPMN **(1981)**	Rat (Wistar,male and female)	OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	Oral (feed) Continuous 13 weeks exposure Approx. 0.5, 2 and 6.7 % Si in the diet	NOEL 6.7 % in feed NOEL highest dose ca. 4000 ≤ 4500 mg/kg bw/day (nominal)	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. 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.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL), 1981	
Substance declared equivalent to **NM-201** (precipitated) Associated characterisation data not available to the WPMN **(1981)**	Rat (Wistar male and female)	90-Day Oral Toxicity in Rodents (OECD TG 408)	Oral (feed) Continuous 13 weeks exposure Approx. 0.5, 2 and 6.7 % Si in the diet	NOEL 6.7 % in feed NOEL highest dose ca. 4000 ≤ 4500 mg/kg bw/day (nominal)	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). 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.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL), 1981	
Substance declared equivalent to **NM-204** (precipitated) Associated characterisation data not available to the WPMN **(1981)**	Rat (Wistar, male and female)	90-Day Oral Toxicity in Rodents (OECD TG 408)	Oral (feed) Continuous 90 day exposure Approx. 0.5, 2 and 6.7 % Si in the diet	NOEL 6.7 % in feed NOEL highest dose ca. 4000 ≤ 4500 mg/kg bw/day (nominal)	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. 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.	Data provided by BIAC. Tests performed by TNO Division of Nutrition and Food Research, Zeist (NL), 1981	

Table 23. Overview of study summaries provided for Repeated Dose Toxicity (chronic)

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/ dose**	**Derived effect value (dose descriptor)**	**Main findings**	**Contributor**
NM-200 (precipitated) (2013)	Rat (male)	Inhalation, subacute inhalation toxicity study, 14d, OECD TG 412	14 d/6h/d 5d/wk 1, 2.5 and 5 mg/m^3 Sampling 1d and 14d post exposure	NOAEC (analytical, not stated in the report) 1.2 mg/m^3 LOAEC (analytical, not stated in the report) 5 mg/m^3	LOAEC based on histopathology of the nasal cavity: hyperplasia of mucous (global) cells (reversible); epithelial hyaline droplets (not reversible) Test particle-related findings were observed only in the nasal cavity and lung. 14d after end of exposure a test particle-related finding was also observed in the lung-associated lymph nodes (LALN). No other organs were affected. NM-200 dust showed a strong acute response, however, rapid recovery upon cessation of exposure.	Data provided by BIAC. Tests performed by Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany
NM-200 (precipitated) (2014)	Rat (male)	Inhalation, subacute inhalation toxicity study, 90d, OECD TG 413	90 d/6h/d 5d/wk 1, 2.5 and 5 mg/m^3	NOAEC (extrapolated) 0.6 mg/m3 LOAEC (analytical), 1 mg/m^3	BAL analysis: Benchmark approach based on PMN increase on 21d post-exposure Histopathology of nasal cavities (day 1 and 91 post-exposure): Hyperplasia (epithelial + mucous cell); epithelial hyaline droplets; epithelial inflammatory cell infiltration	Data provided by BIAC. Tests performed by Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany
Substance declared equivalent to **NM-202** (pyrogenic) Associated characterisation data not available to the WPMN **(1969)**	Rat (Sprague-Dawley female)	Inhalation	6 and 18 weeks and 12 months 5 h/d, 5x/wk initially, but later (time not stated) weekly frequency reduced to 2-3x/wk because suppurative bronchitis and severe inflammation caused losses. Frequency of	No NOAEC identified	RETENTION of silica: After 12-months exposure, about 1 % of administered total respirable dust was estimated to be still retained in the lung. The increase in lung deposition was low from 18 weeks to 12 months of exposure (18 wk: 1.2 mg SiO_2, 12 months: 1.37 mg SiO_2). Mediastinal lymph nodes contained about 0.13 mg SiO_2 after 12 months. After 5 months post-exposure, mean levels of SiO_2 were 0.16 mg/lung and 0.047 mg/lymph node, i.e. a reduction at some 88 % in the lung and more than 50 % in the lymph nodes. PATHOLOGY: Microscopically visible small dust foci could be observed under the pulmonary pleura, mediastinal lymph nodes were moderately enlarged. In the interior of alveoles, numerous macrophages accumulated, partially normal, partially destroyed, associated with deposition of cell debris ("desquamation catarrh"). Perivascular and peribronchiolar small dust	Data provided by BIAC. Tests performed by Institute of Hygiene and Occupational Medicine, Essen (GER) in 1969

			treatment: 5 h/d, 5x/wk, after unspecified time: 2 - 3x/wk 50 - 55 mg/m^3 (total dust) = approx. 30 mg/m^3 (respirable)		foci of macrophages were associated with mild and moderate formation of connective tissue (ranked as grade I to II, based on a ranking system according to Belt&King). In the alveolar septa the collagen formation was increased. In some cases, collagenic fibrosis was detected, partially showing decay. There were no signs of typical silicosis. In the mediastinal lymph nodes, foci and clusters of phagocytes, partially normal, partially showing decay, were observed.	

5.6 Mutagenicity

Table 24 below summarises the outcomes of in vitro mutagenicity studies as reported in literature. Table 25 provides an overview of study summaries for in vitro mutagenicity. Data is provided in bacterial tests by BIAC, (generated before the WPMN set up its testing programme, and it is stated that the material tested is equivalent to the NM-series), mammalian cells (from the Nanogenox project testing the NM-series materials, and from BIAC who submitted data generated before the WPMN set up its testing programme, and it is stated that the material tested is equivalent to the NM-series).

Table 24. Genotoxicity, in vitr testing. Summary of information from scientific literature

Reference	Material / Size	Test Organism / System	Method	Exposure/ dose	Main findings
Genotoxicity – in vitro					
Barnes CA, **2008**	Commercial colloidal and laboratory synthesized silica	Mouse embryonic fibroblast cells	Comet assay	3; 6; 24h 4 and 40 µg/ml	No significant genotoxicity (results were independently validated in two separate laboratories)
Gonzales L., **2010**	Purposely synthesized SAS (2): Stöber SAS (16, 60 and 104 nm)	Human bronchoalveolar carcinoma (A549)	Micronucleus assay (OECD TG 487) Comet assay	40 h (cyto B at t=4h) 45,9 (16 nm SAS) 48,9 (60 nm SAS); 165,3 (104 nm SAS) µg/ml	At non-cytotoxic doses the smallest particles showed a slightly higher fold induction of micronuclei. The authors show that particle number and total surface area appeared to account for micronuclei induction as they both correlated significantly with the amplitude of the effect. Using nominal or cellular dose did not show statistically significant differences. Likewise, alkaline comet assay and FISH-centromeric probing of micronuclei indicated a weak and not statistically significant induction of oxidative DNA damage, chromosome breakage and chromosome loss.

Table 25. Overview of study summaries provided for genotoxicity in vitro

Material (year of study)	Test Organism / System	Method	Exposure/dose	Main findings	Contributor	Comment
Bacterial tests						
Substance declared equivalent to **NM-202** (pyrogenic) Characterisation data not available to the WPMN **(1989)**	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100	Bacterial Reverse Mutation Assay (OECD TG 471)	Exposure duration: no data 667, 1000, 3333, 6667, and 10000 µg/plate ± S9 mix	Negative	Data provided by BIAC. Tests performed by Microbiological Associates, Inc.(USA) in 1989	
Substance declared equivalent to **NM-203** (pyrogenic) Characterisation data not available to the WPMN **(1989)**	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100	Bacterial Reverse Mutation Assay (OECD TG 471)	Exposure duration: no data 667, 1000, 3333, 6667, and 10000 µg/plate ± S9 mix	Negative	Data provided by BIAC. Tests performed by Microbiological Associates, Inc.(USA) in 1989	
Mammalian cells						
NM-200 (precipitated) **(2012)**	Primary rat (Wistar) alveolar macrophage	Comet assay	4 h and 24 h 10, 50, 250 ng/cm² (19, 95, 475 ng/ml) Additionally: 4h at 10 µg/cm² (19 µg/ml) and 24 h at 2.5 µg/cm² (4.75 µg/ml)	Based on the absence of significant increases in the tail intensity in treated BAL cells, as compared to the negative control, NM-200 did not show a significant clastogenic potential or a potential to induce oxidative DNA base lesions.	Data provided by BIAC. Tests performed by Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) (GER) in 2012	No oxidative DNA damage detected with DNA-glycosylase 1 (hOGG1)
NM-200 (precipitated) **(2013)**	Human bronchial 16-HBE cells	Comet assay	3 h and 24 h 5; 20; 40; 80; 100 µg/ml (1.31; 5.26;10.5; 21; 26.3 µg/cm²)	Positive at 3 h: dose-dependent increase Negative at 24 h	Data provided by Nanogenotox performed by UAB (Spain) in 2013	FpG-modified comet assay: negative at 3 h and 24 h

NM-200 (precipitated) **(2013)**	Human bronchial 16-HBE cells	Micronucleus assay (OECD TG 487)	41 h 8; 16; 32 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	Micronucleus assay without cytochalasin B
NM-200 (precipitated) **(2013)**	Human bronchial BEAS-2B cells	Comet assay	3 h 2.56; 25.6; 256; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Positive at 25.6 and 256 µg/ml	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: equivocal response (positive at one dose)
NM-200 (precipitated) **(2013)**	Human bronchial BEAS-2B cells	Micronucleus assay (OECD TG 487)	48 h 8; 16; 32; 64;128 µg/ml (4; 8; 16; 32; 64 µg/cm²)	Negative	Data provided by Nanogenotox performed by FIOH (FL) in 2013	Cytochalasin B added 6 h after NM
NM-200 (precipitated) **(2013)**	Human pulmonary A549 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Equivocal at 3h Negative at 24 h	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: negative at 3 h and 24 h
NM-200 (precipitated) **(2013)**	Human pulmonary A549 cells	Micronucleus assay (OECD TG 487)	48 h 32; 64; 128; 256; 512 µg/ml (6.1; 12.2; 24.4; 48.8; 97.6 µg/cm²)	Negative (experiment 1 and 2)	Data provided by Nanogenotox performed by INRS (F) in 2013	Cytochalasin B added 6 h after NM
NM-200 (precipitated) **(2013)**	Human intestinal Caco-2 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Positive at 3h: 2 doses (25.6 and 256 µg/ml) Positive at 24h: 2 doses (256 and 512 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: equivocal at 3 h and positive at 24 h
NM-200 (precipitated) **(2013)**	Human intestinal Caco-2 cells	Micronucleus assay (OECD TG 487)	52 h 9.5; 28; 85; 128; 256 µg/ml (2.5 ; 7.5 ; 22 ; 34 ; 67 µg/cm²)	Positive in 2 out 3 experiments: dose-dependent increase (85, 128, 256 µg/ml)	Data provided by Nanogenotox performed by Anses (F) in 2013	Cytochalasin B added 24 h after NM
NM-200 (precipitated) **(2013)**	Human primary peripheral blood	Micronucleus assay (OECD TG 487)	30 h 64; 128; 256 µg/ml	Negative	Data provided by Nanogenotox performed by INSA (PT) in 2013	Cytochalasin B added 6 h after NM

	lymphocytes					
NM-200 (precipitated) **(2013)**	L5178Y TK +/-mouse lymphoma cells	In vitro mammalian cell gene mutation tests (OECD TG 476)	24 h 32; 64; 128; 256/ 625; 1250; 2500; 5000 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	
NM-200 (precipitated) **(2012)**	L5178Y TK +/-mouse lymphoma cells	In vitro mammalian cell gene mutation tests (OECD TG 476)	4 h ± S9 mix 10; 100; 300; 900; 2700; 5000 µg/ml	Negative	Data provided by BIAC. Tests performed by Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) (GER) in 2012	Incipient toxicity at 2700 µg/ml, marked at 5000 µg/ml
NM-200 (precipitated) **(2012)**	Chinese hamster lung fibroblasts (V79)	In Vitro Mammalian Chromosome Aberration Test (OECD TG 473)	4 h + S9 mix: 600; 1000; 1500 µg/ml 4 h –S9 mix: 100; 200; 600; 1800 µg/ml 24 h –S9 mix: 2; 5; 16; 48 µg/ml	Negative	Data provided by BIAC. Tests performed by Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) (GER) in 2012	Marked cytotoxicity: ≥ 500 mg/ml (4 h – S9 mix) > 1000 µg/ml (4 h – +S9 mix) ≥ 10 µg/ml (24 h – S9 mix)
NM-201 (precipitated) **(2013)**	Human bronchial 16-HBE cells	Comet assay	3 h and 24 h 5; 10; 20; 40; 60 µg/ml (1.3; 2.63; 5.3; 10.4; 15.8 µg/cm²)	Negative at 3 h and 24 h	Data provided by Nanogenotox performed by UAB (SP) in 2013	FpG-modified comet assay: negative at 3 h and 24 h
NM-201 (precipitated) **(2013)**	Human bronchial 16-HBE cells	Micronucleus assay (OECD TG 487	41 h 32; 64; 128; 256 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	Micronucleus assay without cytochalasin B
NM-201 (precipitated) **(2013)**	Human bronchial BEAS-2B cells	Comet assay	3 h 2.56; 25.6; 256; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Equivocal at 3 h: increase in the % Tail DNA at 1 dose (256 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013	
NM-201	Human	Micronucleus	48 h	Negative	Data provided by	Cytochalasin B

(precipitated) **(2013)**	bronchial BEAS-2B cells	assay (OECD TG 487	4; 8; 16; 32; 64 µg/ml (2; 4; 8; 16; 32 µg/cm²)		Nanogenotox performed by FIOH (FL) in 2013	added 6 h after NM
NM-201 (precipitated) **(2013)**	Human pulmonary A549 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Positive at 3h: at 2 doses (256 and 512 µg/ml) Equivocal at 24h: increase at the lowest dose (2.56 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: negative at 3 h and equivocal at 24 h
NM-201 (precipitated) **(2013)**	Human pulmonary A549 cells	Micronucleus assay (OECD TG 487	48 h 32; 64; 128; 256; 512 µg/ml (6.1; 12.2; 24.4; 48.8; 97.6 µg/cm²)	Positive: (32, 64, 128 and 256 µg/ml), 1st experiment) 256 and 512 µg/ml (2nd experiment)	Data provided by Nanogenotox performed by INRS (F) in 2013	Cytochalasin B added 6 h after NM
NM-201 (precipitated) **(2013)**	Human intestinal Caco-2 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Negative at 3 h Equivocal at 24 h: one dose (25.6 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013 Nanogenotox	FpG-modified comet assay: negative at 3 h and positive at 24 h
NM-201 (precipitated) **(2013)**	Human intestinal Caco-2 cells	Micronucleus assay (OECD TG 487	52 h 9.5; 28; 85; 128; 256 µg/ml (2.5 ; 7.5 ; 22 ; 34 ; 67 µg/cm²)	Positive in 2 out 3 experiments: dose-dependent increase (128, 256 µg/ml exp 1; 28, 85; 128; 256 exp 2)	Data provided by Nanogenotox performed by Anses (F) in 2013	Cytochalasin B added 24 h after NM
NM-201 (precipitated) **(2013)**	Human primary peripheral blood lymphocytes	Micronucleus assay (OECD TG 487	30 h 64; 128; 256 µg/ml	Negative	Data provided by Nanogenotox performed by INSA (PT) in 2013	Cytochalasin B added 6 h after NM
NM-201 (precipitated) **(2013)**	L5178Y TK +/-mouse lymphoma cells	In vitro mammalian cell gene mutation tests (OECD TG 476)	24 h 32; 64; 128; 256/ 625; 1250; 2500; 5000 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	
NM-202 (pyrogenic) **(2013)**	Human bronchial 16-HBE cells	Comet assay	3 h and 24 h 5; 10; 20; 40; 80 µg/ml (1.31; 2.63; 5.3; 10.4; 21 µg/cm²)	Negative at 3 h and 24 h	Data provided by Nanogenotox performed by UAB (SP) in 2013	FpG-modified comet assay: negative at 3 h and

						24 h
NM-202 (pyrogenic)	Human bronchial 16-HBE cells	Micronucleus assay (OECD TG 487	41 h 32; 64; 128 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	Micronucleus assay without cytochalasin B
NM-202 (pyrogenic) **(2013)**	Human bronchial BEAS-2B cells	Comet assay	3 h 2.56; 25.6; 256; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Positive at 3 h: at 3 doses (2.56, 25.6 and 256 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: positive at 3 h
NM-202 (pyrogenic) **(2013)**	Human bronchial BEAS-2B cells	Micronucleus assay (OECD TG 487	48 h 4; 8; 16; 32; 64 µg/ml (2; 4; 8; 16; 32 µg/cm²)	Negative	Data provided by Nanogenotox performed by FIOH (FL) in 2013	Cytochalasin B added 6 h after NM
NM-202 (pyrogenic)	Human pulmonary A549 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Positive at 3h: increase in at 2 doses (25.6 and 256 µg/ml) Equivocal at 24h: increase at one dose	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: positive at 3 h and negative 24 h
NM-202 (pyrogenic) **(2013)**	Human pulmonary A549 cells	Micronucleus assay (OECD TG 487	48 h 32; 64; 128; 256; 512 µg/ml (6.1; 12.2; 24.4; 48.8; 97.6 µg/cm²)	Positive: 64, 128, 256 and 512 µg/ml (1st experiment) 128,256 and 512 (2nd experiment)	Data provided by Nanogenotox performed by INRS (F) in 2013	Cytochalasin B added 6 h after NM
NM-202 (pyrogenic) **(2013)**	Human intestinal Caco-2 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Equivocal at 3 h: one dose (25.6 µg/ml) Equivocal at 24 h: one dose (25.6 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: positive at 3 h and negative at 24 h
NM-202 (pyrogenic)	Human intestinal Caco-2 cells	Micronucleus assay (OECD TG 487)	52 h 9.5; 28; 85; 128; 256 µg/ml (2.5 ; 7.5 ; 22 ; 34 ; 67 µg/cm²)	Positive in 2 out 3 experiments: dose-dependent increase	Data provided by Nanogenotox performed by Anses (F) in 2013	Cytochalasin B added 24 h after NM
NM-202 (pyrogenic) **(2013)**	Human primary peripheral blood	Micronucleus assay (OECD TG 487)	30 h 64; 128; 256; 312.5; 625; 1250 µg/ml	Negative	Data provided by Nanogenotox performed by INSA (PT) in 2013	Cytochalasin B added 6 h after NM

	lymphocytes					
NM-202 (pyrogenic) **(2013)**	L5178Y TK +/-mouse lymphoma cells	In vitro mammalian cell gene mutation tests (OECD TG 476)	24 h 32; 64; 128; 256/ 625; 1250; 2500; 5000 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	
Substance declared equivalent to **NM-202** (pyrogenic) Characterisation data not available to the WPMN **(1990)**	Chinese hamster Ovary (CHO)	In vitro mammalian cell gene mutation Test (OECD TG 476)	5 h -S9 : 10, 50, 100, 150 and 250 µg/ml + S9 : 100, 200, 300, 400 and 500 µg/ml	Negative	Data provided by BIAC performed by Microbiological Associates, Inc., (USA) in 1990	
Substance declared equivalent to **NM-202** (pyrogenic) Characterisation data not available to the WPMN **(1990)**	Chinese hamster Ovary (CHO)	In vitro mammalian cell gene mutation Test (OECD TG 473)	-S9: 18 h 38, 75, 150, 300 µg/ml +S9: 2 h 250, 500, 750, 1000 µg/ml	Negative Cell proliferation began to be inhibited at 30 µg/L (-S9) and 300 µg/L (+S9). Simultaneously, the cell cycle became retarded with an accumulation of cells in the M1 phase. Neither in the control nor in the treated cultures, cells were observed in the M3 phase (except 1 instance in the DMSO control).	Data provided by BIAC performed by Microbiological Associates, Inc., (USA) in 1990	
Substance declared equivalent to **NM-202** (pyrogenic) Characterisation data not available to the WPMN **(1989)**	Primary rat hepatocytes	DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells (OECD TG 482)	18 h to 20 h 10, 30, 100, 300 and 1000 µg/ml	Negative	Data provided by BIAC performed by Microbiological Associates, Inc., (USA) in 1989	
NM-203 (pyrogenic)	Human bronchial 16-	Comet assay	3 h and 24 h 5; 10; 20; 50; 80 µg/ml (1.31;	Negative at 3 h and 24 h	Data provided by Nanogenotox performed by	FpG-modified comet assay:

(2013)	HBE cells		2.63; 5.3; 10.4; 21 µg/cm²)		UAB (SP) in 2013	negative at 3 h and 24 h
NM-203 (pyrogenic) **(2013)**	Human bronchial 16-HBE cells	Micronucleus assay (OECD TG 487)	41 h 8; 12; 16 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	Cytochalasin B added 6 h after NM
NM-203 (pyrogenic) **(2013)**	Human bronchial BEAS-2B cells	Comet assay	3 h 2.56; 25.6; 256; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Positive at 3 h: at 3 doses (2.56, 25.6 and 256 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: positive
NM-203 (pyrogenic) **(2013)**	Human bronchial BEAS-2B cells	Comet assay	24 h 8; 32; 64 µg/ml (4; 16; 32 µg/cm²)	Negative in 3 experiments Positive in 3 experiments	Round robin test Data provided by Nanogenotox in 2013	
NM-203 (pyrogenic) **(2013)**	Human bronchial BEAS-2B cells	Micronucleus assay (OECD TG 487)	48 h 4; 8; 16; 32; 64 µg/ml (2; 4; 8; 16; 32 µg/cm²)	Equivocal: increase at one dose (8 µg/ml)	Data provided by Nanogenotox performed by FIOH (FL) in 2013	Cytochalasin B added 6 h after NM
NM-203 (pyrogenic) **(2013)**	Human bronchial BEAS-2B cells	Micronucleus assay (OECD TG 487	48 h 8; 32; 64 µg/ml (4; 16; 32 µg/cm²)	Negative in 3 experiments Positive in 2 experiments Equivocal in 1 experiment	Round robin test Data provided by Nanogenotox in 2013	Cytochalasin B added 6 h after NM
NM-203 (pyrogenic) **(2013)**	Human pulmonary A549 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Negative at 3h Positive at 24h: increase at 2 doses (25.6 and 256 µg/ml	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: negative at 3 h and positive at 24 h
NM-203 (pyrogenic) **(2013)**	Human pulmonary A549 cells	Micronucleus assay (OECD TG 487)	48 h 32; 64; 128; 256; 512 µg/ml (6.1; 12.2; 24.4; 48.8; 97.6 µg/cm²)	Negative (1st experiment) Equivocal (2nd experiment)	Data provided by Nanogenotox performed by INRS (F) in 2013	Cytochalasin B added 6 h after NM
NM-203 (pyrogenic) **(2013)**	Human intestinal Caco-2 cells	Comet assay	3 h and 24 h 2.56; 25.6; 256 ; 512 µg/ml (1.42; 14.2;142; 284 µg/cm²)	Positive at 3h: 2 doses (2.56 and 25.6 µg/ml) Positive at 24h: 2 doses (25.6 and 512 µg/ml)	Data provided by Nanogenotox performed by IPH (B) in 2013	FpG-modified comet assay: positive at 3 h and equivocal at 24 h

NM-203 (pyrogenic) **(2013)**	Human intestinal Caco-2 cells	Comet assay	24 h 64; 128; 256 µg/ml (32; 64; 128 µg/cm²)	Negative in 3 experiments Positive in 2 experiments	Round robin test Data provided by Nanogenotox in 2013	
NM-203 (pyrogenic) **(2013)**	Human intestinal Caco-2 cells	Micronucleus assay (OECD TG 487)	52 h 9.5; 28; 85; 128; 256 µg/ml (2.5 ; 7.5 ; 22 ; 34 ; 67 µg/cm²)	Positive in 2 out 3 experiments: dose-dependent increase	Data provided by Nanogenotox performed by Anses (F) in 2013	Cytochalasin B added 24 h after NM
NM-203 (pyrogenic) **(2013)**	Human intestinal Caco-2 cells	Micronucleus assay (OECD TG 487)	48 h 64; 128; 256 µg/ml (32; 64; 128 µg/cm²)	Negative in 3 experiments Positive in 3 experiments	Round robin test Data provided by Nanogenotox in 2013	Cytochalasin B added 24 h after NM
NM-203 (pyrogenic) **(2013)**	Human primary peripheral blood lymphocytes	Micronucleus assay (OECD TG 487)	30 h 256; 312.5; 625; 1250 µg/ml	Negative	Data provided by Nanogenotox performed by INSA (PT) in 2013	Cytochalasin B added 6 h after NM
NM-203 (pyrogenic) **(2013)**	L5178Y TK +/-mouse lymphoma cells	In vitro mammalian cell gene mutation tests (OECD TG 476)	24 h 32; 64; 128; 256/ 625; 1250; 2500; 5000 µg/ml	Negative	Data provided by Nanogenotox performed by IPL (F) in 2013	
Substance declared equivalent to **NM-203** (pyrogenic) Characterisation data not available to the WPMN **(1990)**	Chinese hamster Ovary (CHO)	In vitro mammalian cell gene mutation Test (OECD TG 476)	5 h -S9 : 10, 50, 100, 150 and 250 µg/ml + S9 : 100, 200, 300, 400 and 500 µg/ml	Negative	Data provided by BIAC performed by Microbiological Associates, Inc., (USA) in 1990	
Substance declared equivalent to **NM-203** (pyrogenic) Characterisation data not available	Chinese hamster lung fibroblasts (V79)	In Vitro Mammalian Chromosome Aberration Test (OECD TG 473)	-S9: 18 h 38, 75, 150, 300 µg/ml +S9: 2 h	Negative Cell proliferation began to be inhibited at 30 µg/L (-S9) and 300 µg/L (+S9). Simultaneously, the cell cycle became	Data provided by BIAC performed by Microbiological Associates, Inc., (USA) in 1990	

to the WPMN **(1990)**			250, 500, 750, 1000 µg/ml	retarded with an accumulation of cells in the M1 phase. Neither in the control nor in the treated cultures, cells were observed in the M3 phase (except 1 instance in the DMSO control).		
Substance declared equivalent to **NM-203** (pyrogenic) Characterisation data not available to the WPMN **(1990)**	Primary rat hepatocytes	DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells (OECD TG 482)	18 h to 20 h 10, 30, 100, 300 and 1000 µg/ml	Negative	Data provided by BIAC performed by Microbiological Associates, Inc., (USA) in 1989	

Below is given an overview of the scientific literature reporting on in vivo genotoxicity testing of amorphous silicon dioxide, see Table 26, and the testing performed with the NM-series materials in the Nanogenotox project for this end-point, see Table 27.

Table 26. Genotoxicity, in vivo testing. Summary of information from scientific literature

Reference	Material / Size	Test Organism / System	Method	Exposure/ dose	Main findings
Genotoxicity – in vivo					
Johnston CJ, Driscoll KE, Finkelstein JN et al., **2000**	NM-203	Rat (male)	Inhalation ex-vivo / in-vitro HPRT assay in alveolar epithelial cells.	6 h/d, 5 d/wk for 90 days 50 mg/m^3 Basis nominal conc. 50.4 +-19 mg/m^3 Basis analytical conc.	No cytotoxic effects Negative Alveolar type-II cells isolated from the 50-mg/m3 rat group showed no increased mutation frequency as compared to the control.

Downs et al., **2012**	Colloidal silica Levasil from HC Stark Lev 50 – diameter 55nm, specific surface area 50 m^2/g Lev 200-15 nm, specific surface area 200 m^2/g	Rat (male)	Intravenous 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474) 3- Inflammation	3 injections on 3 consecutive days Sampling 4 h after the last injection 25, 50, 125 mg/kg	DNA damage in liver and increase in the % MN-RETs with Lev 15 nm at 50 mg/kg, inflammation (cytokine release in plasma). "Intravenous injection of a 50 mg/kg dose of the 15 nm silica NPs resulted in a small increase in DNA damage in liver and lung tissue, and in white blood cells. No such effect was observed at the lower dose of the same particles or for the 55 nm silica particles, both of which also demonstrated lower acute toxicity than the high dose of the 15 nm silica NPs, which was the maximal dose the animals would tolerate. The small increase in DNA damage for the 50 mg/kg dose of the 15 nm silica NPs was reproduced in an independent second study, in which we also increased the dose of the larger 55 nm silica particles from 25 mg/kg to 125 mg/kg. At this higher dose, now representing the MTD, a 1.5–1.7-fold increase in DNA damage in the liver was also now measured in the 55 nm silica particle treatment group. Very good insight into the toxicity triggered by intravenous treatment of the rats with silica NPs came from histopathological analysis of the livers of the treated animals. Whereas vehicle control animals harboured minimal mononuclear cell infiltration and neutrophilic infiltration, an induction of mononuclear cell infiltrate, increase in Kupffer cell mitotic figures, hepatocellular necrosis, and haemorrhage were observed in the silica NP dose groups showing genotoxic activity. These findings demonstrate an inflammatory reaction of variable degree. Silica NP treatment resulted in an increase in the plasma levels of both markers; the effect was most pronounced for the 15 nm particles, but the 55 nm silica NPs and the quartz particles also caused an induction. These data suggest that there is a size-dependent induction of inflammatory markers, whereby it is possible that the smaller NPs may be penetrating tissues to a greater degree than the larger NPs, promoting an inflammatory response"

Table 27. Overview of study summaries provided for genotoxicity in vivo

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/dose**	**Main findings**	**Contributor**	**Comment**
NM-200 (precipitated) **(2013)**	Rat (Sprague-Dawley. male)	Intratracheal instillation 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474)	1 administration at 0, 24 and 45 h 3; 6; 12 mg/kg bw/d	1-Changes in BAL fluid cell number and composition may be associated with a toxicological process and an inflammatory response (especially granulocytes influx). Whatever the dose considered, a significant increase of influx of neutrophilic granulocytes was observed in BAL fluid from exposed animals with a dose-dependent trend. An increase of the total cell number was also noticed for all the SAS, but this change was not	Data provided by Nanogenotox performed by INRS (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay:

				significant for the lowest dose of NM-200. Main cell types observed in BAL fluid were macrophages and neutrophilic granulocytes, some lymphocytes were also observed but their frequency and number in SAS exposed animals were not significantly different from the control group. In addition, in this study, the percentage of neutrophilic granulocytes in control (vehicle) group appears higher than that is usually obtained following a single intratracheal instillation and may be considered as an experimental artefact due to repeated animal exposure. Increase in lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and N-acetyl-β-Dglucosaminidase (NAG) activities and protein content are often signs of pulmonary toxicity following pulmonary exposure to particulate matter. A significant increase in the broncho-alveolar fluid of LDH and NAG activities was observed. No change in alkaline phosphatase (ALP) was however observed (Figure 17). 2-Thus, intratracheal instillation did not induce any significant increase of DNA damages in all the organs tested. In all the experiments performed, positive controls (MMS Or ENU) always induced significant DNA strand breaks in the tissues analysed.		Negative in all the tested organs except from spleen (equivocal result)
NM-200 (precipitated) **(2013)**	Rat (Sprague -Dawley. male)	Oral 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474) 3- Micronucleus assay in colon	1 administration at 0, 24 and 45 h 5, 10, 20 mg/kg bw/d	1-Negative in duodenum, colon, blood, bone marrow, liver, kidney, spleen 2-Negative 3-Negative	Data provided by Nanogenotox performed by Anses (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative
NM-201 (precipitated) **(2013)**	Rat (Sprague -Dawley. male)	Intratracheal instillation 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474)	1 administration at 0, 24 and 45 h 3; 6; 12 mg/kg bw/d	1- Changes in BAL fluid cell number and composition may be associated with a toxicological process and an inflammatory response (especially granulocytes influx). Whatever the dose considered, a significant increase of influx of neutrophilic granulocytes was observed in BAL fluid from exposed animals with a dose-dependent trend. An increase of the total cell number was also noticed. Main cell types observed in BAL fluid were macrophages and neutrophilic granulocytes, some lymphocytes	Data provided by Nanogenotox performed by INRS (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative

				were also observed but their frequency and number in SAS exposed animals were not significantly different from the control group. In addition, in this study, the percentage of neutrophilic granulocytes in control (vehicle) group appears higher than that is usually obtained following a single intratracheal instillation and may be considered as an experimental artefact due to repeated animal exposure. Increase in lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and N-acetyl-β-Dglucosaminidase (NAG) activities and protein content are often signs of pulmonary toxicity following pulmonary exposure to particulate matter. For all SAS, a significant increase in the broncho-alveolar fluid of LDH and NAG activities was observed. No change in alkaline phosphatase (ALP) was however observed. 2- Intratracheal instillation did not induce any significant increase of DNA damages in all the organs tested. In all the experiments performed, positive controls (MMS or ENU) always induced significant DNA strand breaks in the tissues analysed.		
NM-201 (precipitated) **(2013)**	Rat (Sprague -Dawley. male)	Oral 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474) 3- Micronucleus assay in colon	1 administration at 0, 24 and 45 h 5, 10, 20 mg/kg bw/d	1-Negative in duodenum, colon, blood, bone marrow, liver, kidney, spleen 2-Negative 3-Negative	Data provided by Nanogenotox performed by Anses (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative
NM-202 (pyrogenic) **(2013)**	Rat (Sprague -Dawley. male)	Intratracheal instillation 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474)	1 administration at 0, 24 and 45 h 3; 6; 12 mg/kg bw/d	1- Changes in BAL fluid cell number and composition may be associated with a toxicological process and an inflammatory response (especially granulocytes influx). Whatever the dose considered, a significant increase of influx of neutrophilic granulocytes was observed in BAL fluid from exposed animals with a dose-dependent trend. An increase of the total cell number was also noticed, but this change was not significant for the lowest dose of NM- 202. Main cell types observed in BAL fluid were macrophages and neutrophilic granulocytes, some lymphocytes were also observed but their frequency and number in SAS exposed animals were not significantly different from the	Data provided by Nanogenotox performed by INRS (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative

				control group. In addition, in this study, the percentage of neutrophilic granulocytes in control (vehicle) group appears higher than that is usually obtained following a single intratracheal instillation and may be considered as an experimental artefact due to repeated animal exposure. Increase in lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and N-acetyl-β-Dglucosaminidase (NAG) activities and protein content are often signs of pulmonary toxicity following pulmonary exposure to particulate matter. A significant increase in the broncho-alveolar fluid of LDH and NAG activities was observed. A significant increase in the BAL fluid of protein content was only observed for NM-202. No change in alkaline phosphatase (ALP) was however observed. 2- Intratracheal instillation did not induce any significant increase of DNA damages in all the organs tested. In all the experiments performed, positive controls (MMS or ENU) always induced significant DNA strand breaks in the tissues analysed.		
NM-202 (pyrogenic) **(2013)**	Rat (Sprague -Dawley. male)	Oral 1-Comet assay 2-Micronucleus assay in bone marrow (OECD TG 474) 3-Micronucleus assay in colon	1 administration at 0, 24 and 45 h 5, 10, 20 mg/kg bw/d	1-Negative in duodenum, colon, blood, bone marrow, liver, kidney, spleen 2-Negative 3-Equivocal: increase at 1 dose (5 µg/ml)	Data provided by Nanogenotox performed by Anses (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative
NM-203 (pyrogenic) **(2013)**	Rat (Sprague -Dawley. male)	Intratracheal instillation 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474)	1 administration at 0, 24 and 45 h 3; 6; 12 mg/kg bw/d	1- Changes in BAL fluid cell number and composition may be associated with a toxicological process and an inflammatory response (especially granulocytes influx). whatever the dose considered, a significant increase of influx of neutrophilic granulocytes was observed in BAL fluid from exposed animals with a dose-dependent trend. An increase of the total cell number was also noticed. Main cell types observed in BAL fluid were macrophages and neutrophilic granulocytes, some lymphocytes were also observed but their frequency and number in SAS exposed animals were not significantly different from the control group. In addition, in this study, the percentage of neutrophilic granulocytes in control (vehicle) group appears higher than that	Data provided by Nanogenotox performed by INRS (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative

				is usually obtained following a single intratracheal instillation and may be considered as an experimental artefact due to repeated animal exposure. Increase in lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and N-acetyl-β-Dglucosaminidase (NAG) activities and protein content are often signs of pulmonary toxicity following pulmonary exposure to particulate matter. A significant increase in the broncho-alveolar fluid of LDH and NAG activities was observed. A significant increase in the BAL fluid of protein content was only observed for NM-203 (highest dose). No change in alkaline phosphatase (ALP) was however observed. 2- Intratracheal instillation did not induce any significant increase of DNA damages in all the organs tested. In all the experiments performed, positive controls (MMS or ENU) always induced significant DNA strand breaks in the tissues analysed.		
NM-203 (pyrogenic) **(2013)**	Rat (Sprague -Dawley. male)	Oral 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474) 3- Micronucleus assay in colon	1 administration at 0, 24 and 45 h 5, 10, 20 mg/kg bw/d	1-Negative in duodenum, colon, blood, bone marrow, liver, kidney, spleen 2-Negative 3- Equivocal: increase at 1 dose (5 µg/ml)	Data provided by Nanogenotox performed by Anses (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative
NM-203 (pyrogenic) **(2013)**	Rat (Sprague -Dawley. male)	Intravenous 1- Comet assay 2- Micronucleus assay in bone marrow (OECD TG 474)	1 administration at 0, 24 and 45 h 5, 10, 20 mg/kg bw/d	The highest dose of intravenous (20 mg/kg) induced animal death (3 out of 6). NM-203 induced a dose dependent: increase of spleen weight, increase of liver damage as determined by liver enzymes (glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT)) released into plasma, thrombocytopenia. In both regular and FpG-modified comet assays, no increase of the percentage of tail DNA intensity was noticed in all the organs tested following intravenous NM-203 treatment. Only a significant induction of micronucleus was obtained at the highest dose (20 mg/kg) However, the induction is weak and the results were obtained only from 3 animals due to the lethality induced at this concentration.	Data provided by Nanogenotox performed by INRS (F) in 2013	Sacrifice 3 h after the last administration FpG-modified comet assay: negative

5.7 Carcinogenicity

Table 28 below summarises the information in scientific literature on studies of carcinogenicity of amorphous silicon dioxide.

Table 28. Carcinogenicity of amorphous silicon dioxide: Summary of information from scientific literature

Reference	Material/ Size	Test Organism / System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
Takizawa Y et al, **1988**	Syloid 244	B6C3F1 Mice (male and female)	Oral (feed) OECD TG 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	93 weeks continuous exposure with interim kill after 6 and 12 months 1.25, 2.5 and 5 % (nominal conc.)	NOAEL (highest dose level tested: 5 % in the diet) Effect level ca. 5000 — ca. 7000 mg/kg bw/day (actual dose received)	Negative
Takizawa Y et al, **1988**	Syloid 244	Fischer 344 rat (male and female)	Oral (feed) OECD TG 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	103 weeks continuous exposure with interim kill after 6 and 12 months (10 animals each) 1.25, 2.5 and 5 %	NOAEL (highest dose level tested: 5 % in the diet) Effect level ca. 1800 — ca. 3000 mg/kg bw/day (actual dose received)	Negative

5.8 Toxicity for reproduction

Table 29 below summarises the test data available for toxicity to reproduction. The data is provided by BIAC and results are provided both for the NM-series as well as data generated before the WPMN set up its testing programme, for which it is stated that the material tested is equivalent to the NM-series.

Table 29. Overview of study summaries provided for toxicity for reproduction

Material (year of study)	**Test Organism / System**	**Method**	**Exposure/dose**		**Main findings**	**Contributor**	**Comment**
NM-200 (precipitated) **(2012)**	Rat (Wistar, male and female)	Two-Generation Reproduction Toxicity Study (OECD TG 416)	Oral (gavage), 1x/day F0-Generation: The female animals were dosed during a 10-week premating period and during mating, gestation and lactation up to postnatal day 21. F1-Generation: Selected F1-generation pups were dosed from postnatal day 22 until the day prior to sacrifice.	NOAEL= 1000 mg/kg bw/d (highest tested dose)	No adverse effect on the reproductive performance of rats or on the growth and development of the offspring into adulthood, examined over two consecutive generations.	Data provided by BIAC performed by TNO Triskelion, Zeist (NL) in 2012	
Substance declared equivalent to **NM-202** (pyrogenic) Characterisation data not available to the WPMN **(1963)**	Rat (Wistar male and female)	One-Generation Reproduction Toxicity Study (OECD TG 415)	Oral (diet) Exposure period: 6 months Premating exposure period (males and females): 4.5 months Duration of test: 6 months 497 mg/kg bw (m); 509 mg/kg bw (f)	Generation P NOAEL > 497 mg/kg bw/day Generation F1 NOAEL > 497 mg/kg bw/day	Parents: No clinical symptoms; no mortality, no abnormalities in body-weight gain and feed consumption, no haematological findings. In pups during lactation (total: 45 and 37 (control), resp.), no behavioural or developmental or structural abnormalities.	Data provided by BIAC performed in 1963	No complete one generation study according to current standards: too low number of animals and examinations, one dose only, dose selection unclear (relatively low dose selected).
Substance declared equivalent to	Rat (Wistar male and female)	One-Generation Reproduction Toxicity Study	Oral (diet) 1x/day	Generation P NOAEL > 497 mg/kg bw/day	No adverse effect in parents	Data provided by BIAC performed in	No complete one generation study according to

NM-203 (pyrogenic) Characterisation data not available to the WPMN **(1963)**		(OECD TG 415)	Exposure period: 6 months Premating exposure period (males and females): 4.5 months Duration of test: 6 months 497 mg/kg bw (male); 509 mg/kg bw (female)	Generation F1 NOAEL > 497 mg/kg bw/day		1963	current standards: too low number of animals and examinations, one dose only, dose selection unclear (relatively low dose selected)

5.9 Toxicity in vitro

In vitro Mammalian Toxicology

The in vitro cytotoxicity of Synthetic Amorphous Silica (SAS) is strongly influenced by the chemistry of the surface and in particular by the presence of two surface groups: the silanols (Si-OH) and siloxanes (Si-O-Si). A number of in vitro studies studies on different SAS are available from published literature. The larger the number of silanols and siloxanes groups on the surface of SAS, the greater is the SAS reactivity and thus cytotoxicity. The covering of these active sites by ions will modify the properties of the surface of silicas and can thus lead to a change, and in particular to a reduction, of biological activity. The specific surface of SAS also influences their cellular toxicity. (Chang *et al.*, 2007; Jin *et al.*, 2007; Lin *et al.*, 2006; Lison *et al.*, 2008; Napierska *et al.*, 2009; Sayes *et al.*, 2007). Importantly, one study question the correlation of the results obtained *in vivo* and *in vitro* (Sayes et al., 2007). Although, looking at all the studies collected in Table30, some themes common to what is seen in vivo are emerging: even if SAS is not always shown to be cytotoxic it is quite potent to induce an inflammatory response, most probably mediated via oxidative stress (Lin, W., 2006; Park, EJ, 2009; Choi, SJ, 2009). In one reference various epithelial cells are exposed to dye doped SAS without significant genotoxic effects (Jin *et al.*, 2007). In another study the Comet assay was shown to be reproducibly used to study SAS nanoparticles and no significant genotoxicity on fibroblasts was detected (Barnes *et al.*, 2008), but one study shows also that silica nanoparticles can enter the cell and impair nuclear functions (Chen, 2005).

Table 30. In vitro toxicity testing. Summary from scientific literature

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Toxicity – in vitro					
Lison D, **2008**	Ludox HS-40 Stöber silica nanoparticles 29.3+-4. nm (TEM), 35nm (DLS)	Mouse monocytes (J774), human bronchoalveolar carcinoma (A549), human endothelial cells (EAHY926)	Cytotoxicity (MTT, LDH release, LDH cell content, MTT, and crystal violet staining)	24 h Increasing concentrations of SiO_2 NP in a fixed volume of 200 ll per well or 37 µg/ml dispersed in increasing volumes of DMEM or fixed mass/SA/number of nano-particles (7.5 µg) dispersed in increasing volumes of DMEM	The cellular response was determined by the total mass/number/SA of particles as well as their concentration and it was concluded that the nominal dose remains the most appropriate metric for in vitro toxicity testing of insoluble SiO_2 NP dispersed in aqueous medium.
Napierska D, **2009**	Ludox L-14/L-15 (13.8 nm and 14.7 nm) Stöber silica nanoparticle of 16.4 , 19.4, 60.4 , 104.4 and 335 nm 14, 15, 16, 19, 60, 104 and 335 nm (checked by TEM and DLS)	Human endothelial cells (EAHY926)	Cytotoxicity (MTT, LDH)		It was concluded that that the cytotoxicity of monodisperse amorphous silica nanoparticles with the same morphology was strongly related to particle size. Smaller particles showed significantly higher toxicity than the bigger ones when dose was expressed in mass concentration. The surface area of tested particles was an important parameter determining toxicity of monodisperse amorphous SiO_2 nanoparticles.
Sayes et al., **2007**	Zeofree 80 Aggregates of 1-3 µm (DLS)	Rat lung epithelial cells (L2), rat alveolar macrophages, cocultures of the two	Cytotoxicity (MTT, LDH) Inflammation	1 h to 48 h 0.0052–520lg/cm²	SAS was slightly cytotoxic and induced an inflammatory response in macrophages.
Lin W, et al., **2006**	15 and 46 nm particles from Degussa TEM measurements: 15±5nm 46±12nm	Human bronchoalveolar carcinoma (A549)	Cytotoxicity Oxidative stress (fluorescence)	24; 48; 72h 10, 50, 100 µg/ml	It was concluded that 15-nm and 46-nm SiO_2 nanoparticles significantly reduce cell viability in a dose dependent and time-dependant manner in bronchoalveolar carcinoma-derived cells at 10–100 µg/ml dosage. Both of the SiO_2 nanoparticles showed higher cytotoxicity than the positive control material (Min-U-Sil 5). The ROS generated by exposure to 15-nm SiO_2

					nanoparticles produce oxidative stress in these cells as reflected by reduced GSH levels and the elevated production of MDA and LDH, indicative of lipid peroxidation and membrane damage.
Park E-J, **2009**	12 nm SAS particles from Degussa (Unspecified)	Mouse macrophages (RAW264,7)	Inflammation Oxidative stress (fluorescence)	24 h 5; 10; 20; 40 µg/ml	ROS and pro-inflammatory responses
Chang J.S., Chang L.B., **2007**	Precipitated from Na silicate SEM analysis: 10-15 nm	Human fibroblasts (WS1, CCD-966sk, MRC-5, A549, MKN-28, HT-29)	Cytotoxicity (MTT, LDH)	48 h 667 µg/ml	The cytotoxicity of silica to human cells depends strongly on their metabolic activities but it could be significantly reduced by synthesizing silica with chitosan.
Jin Y, **2007**	Purpose-made dye doped silica 50 nm nanoparticles (from TEOS)	Human bronchoalveolar carcinoma (A549)	Cytotoxicity (MTT)	48 h; 72 h 0.1 µg/ml to 0.5 mg/ml	No significant toxic effects induced by luminescent nanoparticles at the molecular and cellular levels below a concentration of 0.1 mg/mL
Wahl, B, **2008**	Aerosil 200 and unspecified 15 nm silica nanoparticles from Merck	Human intestinal epithelial cells(CaCo-2)	Cytotoxicity (LDH, luminescence assay)		LDH assay showed strong interactions with the tested silica particles. These findings suggest that even well characterized assay systems need a careful evaluation of the particle assay interactions when working with nanoparticles. Furthermore, particles based on the same material exhibit different biological properties depending on whether the material is used in micro- or nanometer range.
Ye, **2010**	SAS colloids from the Center of Analysis and Test Research (Shangai, China) 21, 48 and 86 nm	Human hepatic cells (L-02)	Cytotoxicity (MTT, LDH) Apoptosis Oxidative stress	12, 24, 36 and 48 h 0.2, 0.4 and 0.6 mg/ml	Cytotoxicity in was investigated for size, dose and time dependence. Oxidative stress and apoptosis were induced by exposure to 21 nm SiO_2.
Yang, **2010**	SAS from Wang Jung New Material Co. 13+-3.8 nm 20±3.5 nm	Human keratinocyte cell line (HaCaT)	Proteomic analysis	24 h 2.5 to 80 µg/ml	Size dependent effects on the expression of proteins involved in oxidative stress, cytoskeleton, chaperones, apoptosis, tumour and metabolism is

	50±9.2 nm 365±79 nm (DLS)				demonstrated
Eom, **2009**	Fumed silica (7 nm) Porous silica (5-15 nm) From Sigma	Human bronchial epithelial cell (Beas-2B)	ROS production (fluorescence analysis) Expression of HO-1, Nrf2, NFkB, ikB, ERK, p38 et JNK with western blot	24 h 40 mg/ml	Toxicity via oxidative stress was investigated. Cells exposed to porous silica nanoparticles showed a more sensitive response than those exposed to fumed silica.
Choi SJ, **2009**	Aerosil200 (14 nm)	Human lung epithelial cells (A549 and L-132), human epithelial cells (HeLa) and human osteocarcoma cells (MNNG/HOS)	Cytotoxicity ROS measurement Cytokine expression	72 h 125, 250, 500 µg/ml	Small toxic effects on the proliferation or viability of the cells from the 4 cell lines were observed, but, silica significantly generated ROS, induced release of LDH release and IL-8 production from A549 cells.
Brown SC, **2007**	SAS synthesized for the study (100 and 200 nm spheres and rods)	Human pleural mesothelial *cells* (*Met5A*)	Cytotoxicity (LDH) Inflammation		Increased LDH release and IL-8 expression in the presence of physiological stretch regardless of shape. Moreover, it is evident that shape-induced aggregation may play a significant role in mitigating particle clearance
Lu, **2009**	Purposely prepared mesoporous silica, 30, 50, 110, 170 and 180 nm	Human epithelial cells (HeLa)	Cytotoxicity (MTT) Uptake		Uptake of mesoporous silica by HeLa cells is particle-size-dependent and the maximum uptake by cells occurs at a nanoparticle size of 50 nm. It is expected that the size effect on cell uptake would lead to size-dependent biochemical responses.
Slowing II, **2009**	Purposely prepared meso-porous silica (MSN): 100-300 nm (TEM), ~300nm (DLS) Unspecified SAS from Sigma Aldrich: centred at 459 and 1720 nm (DLS)	Rabbit red blood cells	Haemolysis assay Spectroscopy		The authors show that, contrary to the known cytotoxicity of amorphous silica towards RBCs, MSNs exhibit a high biocompatibility at concentrations adequate for potential pharmacological applications. We demonstrated that the haemolytic properties of MSNs are related to the number of silanol groups accessible to the

					cell membranes of RBCs
Park YH et al.,**2010**	SAS particles from Degussa 7 nm and 10-20 nm (SEM)	Human keratinocytes, human skin equivalent model	Cytotoxicity Irritation		Reduced cell viability no acute cutaneous irritation.
Rabolli V., **2010**	Purposely synthesized SAS (2): - Stöber SAS - Lysin Silica Sols and Ludox HS-40, Ludox LS-30, Ludox SM-30	Mouse monocytes (J774), human endothelial cells (EAHY926), mouse fibroblasts (3T3) and human erythrocytes	Cytotoxicity (MTT and WST1 assay)		In J774 macrophages, the cytotoxic activity increased with external surface area and decreased with micropore volume; in EAHY926 and 3T3 cells, the cytotoxic activity increased with surface roughness and small diameter; in erythrocytes, the haemolytic activity increased with the diameter of the SAS nanoparticle. The article concludes that it is possible to predict with good accuracy the in vitro cytotoxic potential of SNPs on the basis of their physico-chemical characteristics.

5.10 Summary of in vitro testing by Japan on alternate SiO_2 material

Table 31 below summarises the in vitro test results of the alternate material provided by Japan.

Table 31. Summary of in vitro testing by Japan on alternate SiO2 material

NANOMATERIAL INFORMATION/ IDENTIFICATION	**Nanomaterial name:** Silicon Dioxide (SAS) **Nanotek** **CAS Number:** (CAS no. general for SiO2: 7631-86-9) **Structural formula/ molecular structure:** SiO_2, strong, directional covalent bonds, and has a well-defined local structure: four oxygen atoms are arrayed at the corners of a tetrahedron around a central silicon atom **Composition of nanomaterial being tested:** purity: >99.9 % **Basic morphology:** Amorphous, Spherical shape, Specific surface area (BET): 86.0m^2/g, primary particle size (TEM, average): 25 nm **Description of surface chemistry:** Neither coated nor modified. **Major commercial uses:** as it is a High Production Volume (HPV) chemical; car tires (rubber), printing inks, pharmaceuticals, cosmetics, etc. **Known catalytic activity:** None **Method of production:** Physical Vapor Synthesis (PVS) method **Method of detection:**			
***In Vitro* tests**	- Nanotek primary particle size: 25 nm purity: 99.9 % from C. I. Kasei Co. Ltd. (Japan) http://www.cik.co.jp/product/nanotek/english/	Japan/AIST http://www.aist-riss.jp/projects/nedo-nanorisk/rd/iwahashi2009_e.html Contact: t-igarashi@aist.go.jp	Ten different cell lines including A549, HaCaT, and THP-1. Cell viability, oxidative stress, DNA injury, colony forming ability, gene expression of cytokine and apoptosis.	SiO_2 NP induced oxidative stress in cultured cells. The intracellular ROS level was elevated by SiO_2 exposure. Subsequently, cell viability was decreased. The MTT activity was slightly decreased (50 % of untreated cells) at conc. of approx. 50 µg/ml for 24 h exposure. Activity of apoptosis related enzyme caspase-3 was increased by 24 h exposure.

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APPENDIX I: MATERIAL SELECTION

Silicon dioxide (SiO_2) exists in a number of different structural forms, and for the materials selection some background information was needed, and it is outlined below.

Silicon dioxide forms

Silicon dioxide has the general CAS no. 7631-86-9, covering all forms i.e. both crystalline and non-crystalline (amorphous) forms. Amorphous silica forms are subdivided in naturally occurring amorphous silica (diatomite) and synthetic forms. Synthetic amorphous silica (SAS) as defined here is intentionally manufactured and does therefore not contain measurable levels of crystalline silica which causes adverse health effects such as silicosis (Arts et al. ; Merget et al. 625-34).

Synthetic amorphous silica can be divided in two groups according to whether the manufacturing process is by the wet route (precipitated silica, silica gel, colloidal silica, CAS No 112926-00-8) or the thermal route (pyrogenic silica, CAS No 112945-52-5). Colloidal silica (silica sols) is stable dispersions of SASs in a liquid, usually water.

Furthermore, SASs, which are generally hydrophilic, may become hydrophobic after surface treatment. SASs exist as highly pure, white, fluffy powders or milky-white dispersions of these powders in liquids (usually water). As already indicated, the different SASs have different CAS numbers according to the route of production, see figure I.1.

Thus, "synthetic amorphous silica" a general description of the output of chemical manufacturing processes. A specific final product is described e.g. by giving the manufacturing process, the processing plant, trade name and the physical-chemical characteristics. In the following the term "source" will used as denominator for such a specific final product.

Figure I.1. Overview of forms of Silicon Dioxide *[CAS No. in brackets]*

Silicon Dioxide [CAS No. 7631-86-9]		
Synthetic Amorphous Silica [7631-86-9]	**Amorphous Silica** [7631-86-9]	**Crystalline Silica** [7631-86-9]
Wet Route production * Silica gel [112926-00-8] * Precipitated Silica [112926-00-8] **Thermal Route Production** * Pyrogenic Silica [112945-52-5] **Surface modified silica** For example: * [67762-90-7], * 68611-44-9], * [68909-20-6]	**Natural:** * Kieselguhr [61790-53-2] * Calcinated [91053-39-3] * Flux-calcinated [68855-54-9] **By-products** * Fused silica [60676-86-0] * Silica fume [69012-64-2]	* Crystobalite [14464-46-1] * Quartz [14808-60-7] * Tridymite [15468-32-3]

In the OECD Sponsorship Program, the main criteria for selecting a principal material are widespread application(s) combined with a significant market for the material, and the nano status is implicit as the material is proposed under the sponsorship programme. When discussing with BIAC which of the SASs to choose as a principal material, BIAC highlighted that many of the qualities of SAS on the market would be micron scale rather than nano scale due.

In order to select the principal SAS, a further understanding of the structure of SASs was needed and several meetings with ASASPS took place during 2009. The first one was held 12 January 2009, where BIAC highlighted that synthetic amorphous silica is, according to ISO definitions, a nanostructured material consisting of primary nanoparticles which quickly aggregate and agglomerate. In addition, the meeting concluded that little or no material characterization with regard to physical-chemical properties as required under the OECD Sponsorship program was available for the test materials used in the

toxicological and ecotoxicological studies[7] presented on this occasion. Since SAS consists of aggregates with a mean size above the nanoscale, which build up agglomerates with a size range above 100µm, it was not straight forward to decide which SAS material(s) to select as a principal material for the Sponsorship Program and quite some discussion took place, arriving at the following conclusions:

Based on current information available, the aggregates and agglomerates of SASs used in the current main applications are larger than the nano-scale. In addition, while the SAS aggregates and agglomerates are known to be nanostructured, the number of nano-sized aggregates/agglomerates and discrete nanoparticles, which they may contain, is not clear as characterization data are lacking; when measuring the size of SAS as supplied from a manufacturer the measurement would reflect the particle size distribution of the aggregates/agglomerates, with a tail of particles with smaller sizes. Furthermore, the measured size of SAS in dispersion strongly depends on the applied shear forces during sample preparation.

Analysis of some background information

Three main reference reports on silicon dioxide have been identified: the aforementioned reports ECETOC JACC report No. 51 and the OECD HPV report and the SASSI report.

Examining the available information relating to the physical-chemical data presented in these reports, and especially the characterization of size of particles used in the studies shows that none of the reports contain any detailed characterization data with regard to physical-chemical properties as required under the OECD Sponsorship program.

The OECD High Production Volume (HPV) Chemicals report on synthetic amorphous silica and silicates gives a good general description of SAS under the HPV program. An underlying assumption in this report, which was agreed to by OECD under the HPV program, is that the different SASs are sufficiently similar that their data obtained by using OECD test guidelines (TG) for chemicals can be combined into one HPV dataset. As a consequence of this combination of data, the base data set for one source of SAS, as required under the OECD WPMN program, cannot be extracted from the summary information presented. The report contains limited detailed characterization data with regard to the physical-chemical properties as required under the OECD Sponsorship program. In order to possibly use the data for the OECD sponsorship program for nanomaterials it would be necessary to extract and evaluate the substance characterization information, if available, from each original study.

The JACC report, p.12, cites information that the primary particle size is in the range of 0.001 to 0.1 micron, i.e. the primary particles are nano-sized, adding the footnote that "Primary particles do not normally exist as individual units". The report analyses in more detail the size of particles concluding that (p. 38) "Under conditions of normal technical handling and use, agglomerates[8] are the relevant particles, both for pyrogenic and precipitated SAS." Appendix B of the JACC report lists the SAS types mentioned in the report and the list contains 49 hydrophilic SAS, which are the ones of interest for the OECD sponsorship program. The report does not give a detailed material characterization with regard to the physical-chemical properties as required under the OECD Sponsorship program. The JACC report indicates [p. 25] that the different sources of SAS have different particle structures depending on the route of manufacture. In order to possibly use the data for the OECD Sponsorship Program it would be necessary to extract and evaluate the substance characterization information, if available, from each original study.

The SASSI report is essentially based on data presented in the two reports mentioned above and it demonstrates that SAS is a nanostructured material, but does not provide in-depth characterization data for individual sources of SAS with regard to the physical-chemical properties as required under the OECD Sponsorship program. In addition, the SASSI report indicates [page 16] that depending on the manufacturing process, the different sources of SAS differ across several physical-chemical properties, including size and surface area.

It is important, in this context, to note that the OECD WPMN and the OECD HPV programme are two programmes addressing different issues. The OECD HPV programme addresses industrial chemicals

[7] The outcome of those studies are part of the information found the document published by ECETOC, Synthetic amorphous silica, JACC n° 51, ISSN-0773-6339-51, 2006.

[8] In the JACC report, p. 24, the convention is described: "Agglomerates are assemblies of aggregates"

reported to be produced or imported at levels greater than 1,000 tonnes through hazard assessment of those chemicals based on the Screening Information Data Set (SIDS, guideline and overview of tests available at http://www.oecd.org/dataoecd/13/18/36045056.pdf) obtained by applying then current OECD test guidelines. The OECD WPMN agreed an initial list of fourteen representative manufactured nanomaterials and a list of endpoints, which would be addressed for the hazard assessment of those materials. The WPMN then launched the OECD's Sponsorship Programme on the Testing on Manufactured Nanomaterials, to generate this information for the selected manufactured nanomaterials through actual testing, preferably using the OECD test guidelines. Based on the results it is hoped to be possible to evaluate (1) best ways to characterise the physical-chemical properties of nanomaterials, (2) if the OECD test guidelines are applicable to nanomaterials or if modifications or new methods are needed, and (3) how far properties of a nanomaterial differ from the bulk equivalent, if existing, with respect to human health and environmental safety. Thus the two programmes have different scopes, and results from one programme are not necessarily transferable to the other.

An additional souce of information was consulted, the public EU REACH registration dossier, which provides "classical" physic-chemical characterisation and only little information relevant for the end-points under the OECD Sponsorship Program.

The reports mentioned above present information on different nanostructured sources of SAS which are combined into one dataset. The objective of the sponsorship programme is to generate a complete sponsorship programme dataset for one principal material including physical-chemical characterization. For the (eco-) toxicological testing as presented in the above mentioned reports, the substance used in the tests performed is either not mentioned or if mentioned the physical-chemical characterisation data are not provided. The reports thus provide background information giving a general overview of SASs. Under the WPMN's sponsorship programme, the principal SAS needs its own specific data set where the reported test results relate to tests performed using specifically the principal SAS. Alternate SASs will also be tested, at least with regard to the physical-chemical data.

Towards selecting principal material

After several meetings, ASASP suggested a number of materials with different uses and process of production; the colloidal silicon dioxides were then excluded as the liquid phase could contain anti-microbial agents, necessitating a number of additional studies if this would influence the outcome of the (eco)toxicity testing. A number of candidate materials were identified, based on volume and use, see table I.2:

Table I.2. Possible principal material for synthetic amorphous silica.

JRC Reference	Sample Refer.	Use	Production process
NM-200	PR-A-02	Food	precipitated
NM-201	PR-B-01	Rubber	precipitated
NM-202	PY-AB-03	Rubber and Food	pyrogenic
NM-203	PY-A-04	Food	pyrogenic
NM-204	PR-A-05	Food	precipitated

The background reports mentioned above outline a number of differences in the properties of the silicon dioxide obtained through the two production methods: The JACC report describes (p.24-25) that the different routes of manufacture for SAS lead to different particle structures: the precipitation route leads to compact aggregates whereas the pyrogenic route leads to open branch chain aggregates. Another interesting difference is the number of silanol groups (Si-OH) per unit surface area (per nm^2) which varies depending on the manufacturing process and the analytical methods (Zhuravlev, L.T. (2000)). According to the JACC report (p.15) the number of silanol groups (Si-OH) per unit surface area (per nm^2) varies from 5.0 to 5.7 for precipitated silica, to 1.25 to 2.5 for pyrogenic silica. The silanol group is hydrophilic and the solubility of SAS depends on the number of silanol groups per unit surface area, i.e. the solubility of silicon dioxide depends on route of production. The the dissolution process is described in this report in the section on "surface chemistry".

APPENDIX II: CONTACT DETAILS FOR INVOLVED INSTITUTIONS (2014)

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APPENDIX III: ENDPOINTS IN THE WPMN TESTING PROGRAMME

Nanomaterial Information / Identification		**Environmental fate**	
1	Nano material name	27	Dispersion stability in water
2	CAS number	28	Biotic degradability
3	Structural formula / molecular structure	29	- Ready biodegradability
4	Composition of NM being tested (incl. degree of purity, known impurities or additives)	30	- Simulation testing on ultimate degradation in surface water
5	Basic Morphology	31	- Soil simulation testing
6	Description of surface chemistry (e.g. coating or modification)	32	- Sediment simulation testing
7	Major commercial uses	33	- Sewage treatment simulation testing
8	Known catalytic activity	34	Identification of degradation product(s)
9	Method of production (e.g. precipitation, gas phase)	35	Further testing of degradation product(s) as required
Physical-chemical Properties and Material Characterization		36	Abiotic degradability and fate
10	Agglomeration / aggregation	37	- Hydrolysis, for surface modified nanomaterials
11	Water solubility	38	Adsorption- desorption
12	Crystalline phase	39	Adsorption to soil or sediment
13	Dustiness	40	Bioaccumulation potential
14	Crystallite size	41	Bioaccumulation in sediment
15	Representative TEM picture(s)	**Environmental toxicology**	
16	Particle size distribution	42	Effects on pelagic species (short/ long term)
17	Specific surface area	43	Effects on sediment species (short/ long term)
18	Zeta potential (surface charge)	44	Effects on soil species (short/ long term)
19	Surface chemistry (where appropriate)	45	Effect on terrestrial species
20	Photo-catalytic activity	46	Effect on micro-organisms
21	Pour density Must be completed	47	Other relevant information
22	Porosity	**Mammalian toxicology**	
23	Octanol-water partition coefficient, where relevant	48	Pharmacokinetics (ADME)
24	Redox potential	49	Acute Toxicity
25	Radical formation	50	Repeated dose toxicity
26	Other relevant information (where available)		IF AVAILABLE
		51	Chronic toxicity
Material safety		52	Reproductive toxicity
57	Flammability	53	Developmental toxicity
58	Explosivity	54	Genetic toxicity
59	Incompatability	55	Experience with human exposure
		56	Other relevant test data

APPENDIX IV: PARTNERS OF THE JOINT ACTION NANOGENOTOX

List of collaborating partners in NANOGENOTOX:

NANOGENOTOX

• Contact •

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French agency for food, environmental and occupational health & safety

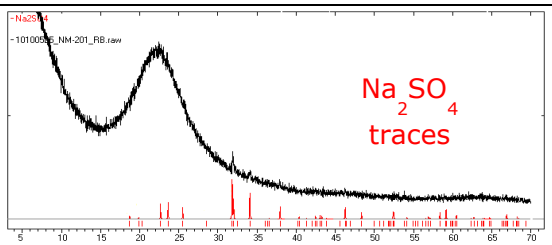
Partners

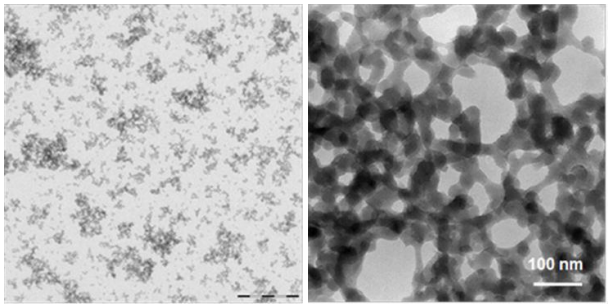
French Agency for Food, Environmental and Occupational Health Safety (France)	ANSES
Federal Institute of Risk Assessment (Germany)	BfR
French Atomic Energy Commission (France)	CEA
Institute of Mineralogy and Crystallography (Bulgaria)	IMC-BAS
Veterinary and Agrochemical Research Centre (Belgium)	CODA-CERVA
Finnish Institute of Occupational Health (Finland)	FIOH
Roumen Tsanev Institute of Molecular Biology Academy of Sciences (Bulgaria)	IMB-BAS
Institut national de recherche et de sécurité (France)	INRS
National Health Institute Doutor Ricardo Jorge (Portugal)	INSA
Scientific Institute of Public Health (Belgium)	IPH
Institut Pasteur of Lille (France)	IPL
Istituto superiore di sanità (Italy)	ISS
The Nofer institute of Occupational Medicine (Poland)	NIOM
National Research Centre for the Working Environment (Denmark)	NRCWE
National Institute for Public Health and the Environment (The Netherlands)	RIVM
Universitat Autònoma de Barcelona (Spain)	UAB

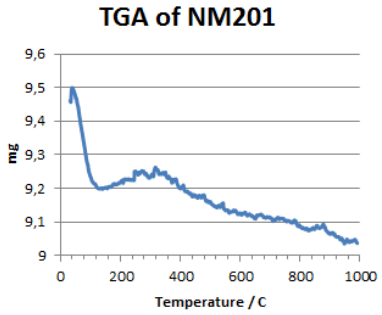
APPENDIX V: OVERVIEW OF CHARACTERISATION RESULTS FOR NM-201, NM-202, NM-203 AND NM-204

The tables in Appendix V are based on Rasmussen et al. 2013. Dispersion, when relevant, was mostly done following the Nanogenotox dispersion protocol. Testing by the JRC may not have used this dispersion protocol.

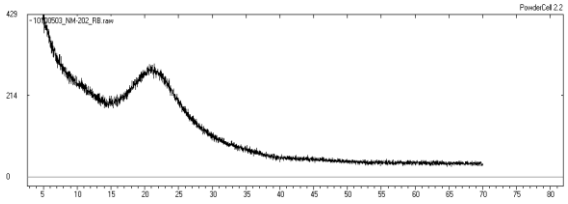
NM-201, summary of physical-chemical characterisation results

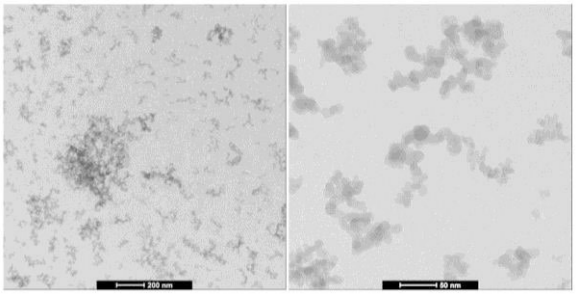
Method	Institution	Results for NM-201
Homogeneity		
DLS		Study not performed
Agglomeration / aggregation		
SAXS	CEA	Structure and size parameters extracted from SAXS data. Gyration radius of primary particles and aggregates $2xRg_1$: 20 nm and $2xRg_2$: 180 nm, fractal dimension D_f: 2.45 and number $N_{part/agg}$ of particles per aggregate: 457
DLS	CEA	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 208.1±34.5, PdI: 0.352±0.028 • Ultra-pure water dispersion (inter vial study) Z-average (nm): 197.0±15.7, PdI: 0.337±0.020
	JRC	• miliQ water dispersion. Z-average (nm): peak 1: 161, peak 2: 968, PdI: 0.420
TEM	CODA-CERVA, IMC-BAS	High porosity nanostructured material which may be considered aggregates of primary particles. Median diamater (nm): 43±4. Feret min: 25.4 nm (median of 5331) Feret max: 34.5 nm (median of 5331) Morphology of aggregates/agglomerates: Medium sphericity, rounded to well--rounded. % of aggegates <100 nm: 81.5%
AFM	CEA	Third dimension of the agglomerates/aggregates: median (of 1275): 33.5 nm
Water Solubility		
24-hour acellular *in vitro* incubation test in special solutions	NRCWE	The 24-hour dissolution ratio of NM-201 was measured in three different media: 0.05% BSA in water, Gambles solution and Caco 2 media. Both NM-201 and the Al impurities are partially soluble in Gambles Solution and Caco2 media but amounts vary considerably with the medium. In 0.05% BSA in water only the Al impurities were partially soluble, Si was below the detection limit. The relative amounts of dissolved Al impurities and dissolved Si differed depending on medium, which suggests different solubility behaviour of the Al impurity and NM-201 depending on the medium.
Crystalline phase		
XRD	JRC	Synthetic amorphous silica. Traces of crystalline material seen around 2-Theta equal to 32° and 34°, which is consistent with the suggested presence of Na_2SO_4
	NRCWE	 Synthetic Amorphous silicon dioxide; impurities of Na_2SO_4.

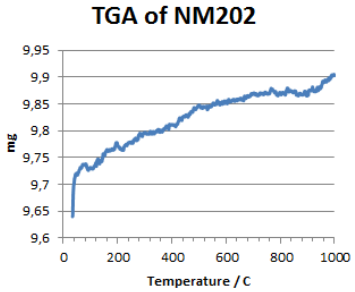
Method	Institution	Results for NM-201
	IMC-BAS	Synthetic amorphous silicon dioxide.
Method	**Institution**	**Results for NM-201**
Dustiness		
Small Rotating Drum	NRCWE	Inhalable dustiness index (n=3) 6034±199 Respirable dustiness index (n=3) 218±24
Vortex Shaker Method	INRS	Respirable dustiness index (n=1) 65000
Crystallite size		
SAXS	CEA	Amorphous material. Equivalent diameter for spheres: 22 nm, gyration radius 2xRg_1 = 20 nm
XRD	JRC	Synthetic amorphous silica. Traces of crystalline material seen around 2-Theta equal to 32° and 34°, which is consistent with the suggested presence of Na_2SO_4
	NRCWE	Synthetic amorphous silicon dioxide. Crystalline impurities of Na_2SO_4.
Representative TEM picture(s)		
TEM	CODA-CERVA, IMC-BAS	 Aggregates with complex, open network structure.
Particle size distribution		
SAXS	CEA	Equivalent diameter for spheres: 22 nm, gyration radius 2xRg_1 = 20 nm
TEM	CODA-CERVA	Primary particle size: 17±8 nm
	IMC-BAS	Primary particle size: 18
	INRS	Primary particle size: 19±4 nm
TEM	CODA-CERVA, IMC-BAS	Number (expressed in %) of SAS NM particles smaller than 100nm, 50nm and 10nm <100 nm - 81.5%, <50 nm – 55.3% <10 nm – 1.1%
DLS	CEA	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. • Ultra-pure water dispersion (intra vial study) Z-average (nm): 208.1±34.5, PdI: 0.352±0.028, FWHM peak width (nm): 140.4±105.7 • Ultra-pure water dispersion (inter vial study) Z-average (nm): 197.0±15.7, PdI: 0.337±0.020 FWHM peak width (nm): 105.6±49.3
	JRC	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. • miliQ water dispersion. Z-average (nm): peak 1: 161, peak 2: 968, PdI: 0.420
CLS	JRC	Peak (nm): 88, half width: 136, CLS Pdl: 2.65
Specific Surface Area		

Method	Institution	Results for NM-201
BET	IMC-BAS	140.46 (m^2/g)
SAXS	CEA	123.3±8.3 (m^2/g)
Method	**Institution**	**Results for NM-201**
Zeta Potential (surface charge)		
Zetametry	CEA	NM-201 forms a stable suspension, with negatively to neutral charged particles. The zeta potential varied greatly as function of pH and reached -40 mV around pH 7. IEP <2
	JRC	Zeta potential at pH 6.9, milliQ water: -51.7 (mV).
Surface Chemistry		
XPS	JRC	The following elements were identified in the surface of NM-201: O (70.3 at%), Si (23.6 at%), C (4.5 at%), Na (1.5 at%), Ce (0.25 at%) and S (0.01 at%). The presence of C is considered to be due to surface contamination from hydrocarbons from air that have attached themselves to the material surface.
TGA	NRCWE	 A significant mass loss is observed below 100°C (water). Gradual 3 wt% mass loss was observed above 110°C indicating e.g. loss of water associated in the micro pores or associated with the presence of Na_2SO_4.
Photo-catalytic activity		
End-point not relevant for SAS		
Pour-density		
Weighing	INRS	0.28 g/cm^3 (8 wt.% water content)
Porosity		
BET	IMC-BAS	Micropore volume (mL/g): 0.00916
Octanol-water partition coefficient		
End-point not relevant		
Redox potential		
OxoDish fluorescent sensor plate for O_2 detection	NRCWE	The evolution of O_2 level during 24-hour incubation was measured in three different media. Different dO_2 values were observed for all applied media. In the Gambles solution and Caco 2 media the concentration of dO_2 was the highest (increased ca. 40 µmol/l) for 0.16mg/ml concentration of NM-201. In the 0.05% BSA in water the dO_2 level increases along with the concentration of NM-201 The results suggest that NM-201 has oxidative behaviour in these incubation media.
Radical formation		
HPLC + UV	NRCWE	Using the benzoic acid probe to form 4 hydroxy benzoic acid in a phosphate buffered hydrous solution, gave no detectable concentration OH radicals.
Composition		
ICP-OES	CODA-CERVA	>0.01%: Al(>0.1%), Ca, Na(>0.1%), S; 00.5-0.01%: Zr; 0.001-0.005%: Fe, K, Mg
EDS	IMC-BAS	Na-4400ppm, Al- 7400ppm, S- 4600ppm, Si -45.27 (wt %), O (wt%) calculated- 53.08

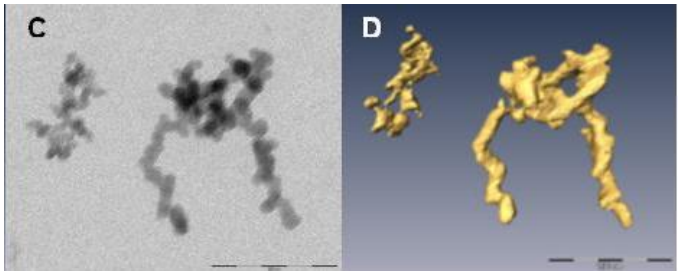
NM-202, summary of physical-chemical characterisation results

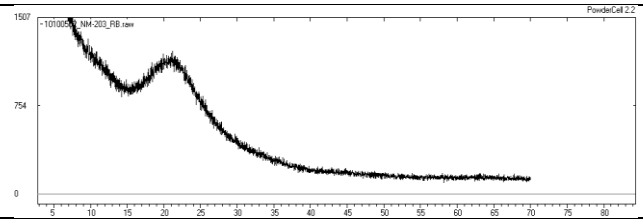
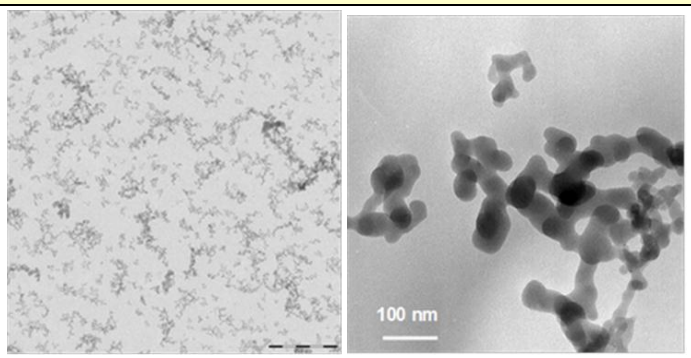
Method	Institution	Results for NM-202
Homogeneity		
DLS		Study not performed.
Agglomeration / aggregation		
SAXS	CEA	Structure and size parameters extracted from SAXS data. Gyration radius of primary particles and aggregates Rg_1: 16 nm and Rg_2: 100 nm, fractal dimension D_f: 2.5 and number $N_{part/agg}$ of particles per aggregate: 200
DLS	CEA	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 175.9±4.5, PdI: 0.355±0.001
	JRC	• miliQ water dispersion. Z-average (nm): peak 1: 156, peak 2: 200, PdI: 0.160
TEM	CODA-CERVA, IMC-BAS	High porosity nanostructured material which may be considered aggregates of primary silicon dioxide particles. Median diamater (nm): 53±9. Feret min: 37.2 nm (median of 4248) Feret max: 58.4 nm (median of 4248) Morphology of aggregates/agglomerates: Low sphericity—very angular to sub-angular. % of aggregates <100 nm: 80.4 %
AFM	CEA	Third dimension of the agglomerates/aggregates: median (of 1103): 38.2 nm
Water Solubility		
24-hour acellular *in vitro* incubation test in special solutions	NRCWE	The 24-hour dissolution ratio of NM-202 was measured in three different media: 0.05% BSA in water, Gambles solution and Caco 2 media. Both NM-202 and the Al impurities are partially soluble in all media but amounts vary considerably with medium as does the relative amounts of dissolved Al impurities compared with dissolved Si suggesting different solubility behaviour of the Al impurity and NM-202 depending on the medium.
Crystalline phase		
XRD	JRC	Synthetic amorphous silicon dioxide.
	NRCWE	 Synthetic amorphous silicon dioxide.
	IMC-BAS	Synthetic amorphous silicon dioxide.
Dustiness		
Small Rotating Drum	NRCWE	Inhalable dustiness index (n=3) 4988±1866 Respirable dustiness index (n=3) 91±11
Vortex Shaker Method	INRS	Respirable dustiness index (n=1) 510000

Method	Institution	Results for NM-202
Crystallite size		
SAXS	CEA	Amorphous material. Equivalent diameter for spheres: 22 nm, gyration radius $2xRg_1$ = 15 nm
XRD	JRC	Synthetic amorphous silicon dioxide.
	NRCWE	Synthetic amorphous silicon dioxide.
Representative TEM picture(s)		
TEM	CODA-CERVA, IMC-BAS	 Aggregates with complex, open network structure.
Particle size distribution		
SAXS	CEA	Equivalent diameter for spheres: 22 nm, gyration radius $2xRg_1$ = 15 nm
TEM	CODA-CERVA	Primary particle size: 15±7 nm
	IMC-BAS	Primary particle size: 20 nm
	INRS	Primary particle size: 18±3 nm
TEM	CODA-CERVA, IMC-BAS	Number (expressed in %) of SAS NM particles smaller than 100nm, 50nm and 10nm <100 nm - 80.4%, <50 nm – 55% <10 nm – 0.9%
DLS	CEA	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. • Ultra-pure water dispersion (intra vial study) Z-average (nm): 175.9±4.5, PdI: 0.355±0.001, FWHM peak width (nm): 56.2±2.9
	JRC	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. • miliQ water dispersion. Z-average (nm): peak 1: 156, peak 2: 200, PdI: 0.160
CLS	JRC	Peak (nm): 73, half width: 45, CLS Pdl: 1.43
Specific Surface Area		
BET	IMC-BAS	204.11 (m^2/g)
SAXS	CEA	184±17.8 (m^2/g)
BET	JRC	Sample stored at 40°C: single point: 186.5392 (m^2/g); multi point: 191.9871 (m^2/g). Sample stored at -80°C: single point: 187.4781 (m^2/g); multi point: 192.9282 (m^2/g).

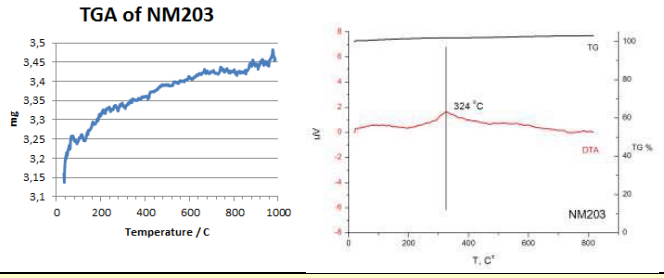
Method	Institution	Results for NM-202
Zeta Potential (surface charge)		
Zetametry	CEA	NM-202 forms a stable suspension, with negatively to neutral charged particles. The zeta potential, however, varied greatly as function of pH and reached -40 mV around pH 7. IEP 2-4.
	JRC	Zeta potential at pH 6.5, milliQ water: -43.7 (mV).
Surface Chemistry		
XPS	JRC	The following elements were identified in the surface of NM-202: O (72.1 at%), Si (25.0 at%) and C (2.9 at%). The presence of C is considered to be due to surface contamination from hydrocarbons from air that have attached themselves to the material surface.
TGA	NRCWE	 No mass loss observed. Observed mass-increase in graph is due to boyancy.
Photo-catalytic activity		
End-point not relevant for SAS		
Pour-density		
Weighing	INRS	0.13 g/cm^3 (1 wt.% water content)
Porosity		
BET	IMC-BAS	Micropore volume (mL/g): 0.0.00084
Octanol-water partition coefficient		
End-point not relevant		
Redox potential		
OxoDish fluorescent sensor plate for O_2 detection	NRCWE	The evolution of O_2 level during 24-hour incubation was measured in three different media. Different dO_2 values were observed for all applied media. In the Caco 2 media the concentration of dO_2 was the highest for 0.16mg/ml concentration of NM-202. In the Gambles solution and in 0.05% BSA in water the dO_2 level increased along with the concentration of NM-202. Maximum increase of ca. 30 µmol/l was observed in 0.05% BSA water. The results suggest that NM-202 has oxidative behaviour in these incubation media.
Radical formation		
HPLC + UV	NRCWE	Using the benzoic acid probe to form 4 hydroxy benzoic acid in a phosphate buffered hydrous solution, gave no detectable concentration OH radicals.
Composition		
ICP-OES	CODA-CERVA	No impurities detected
EDS	IMC-BAS	Al- 4500 ppm, Ca- 1800 ppm, Si -46.23 (wt %), O (wt%) calculated- 53.14

NM-203, summary of physical-chemical characterisation results

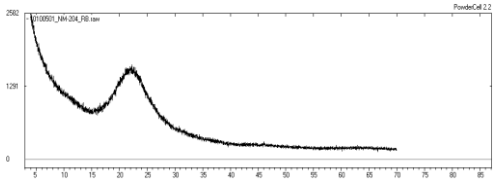
Method	Institution	Results for NM-203
Homogeneity		
DLS	CEA, INRS, NRCWE	Repeated DLS studies were performed between different vials and from different areas within vials. The observed variability between the different vials is very low (2-3%) but intra-vial is high, ca. 20%.
Agglomeration / aggregation		
SAXS	CEA	Structure and size parameters extracted from SAXS data: Gyration radius of primary particles and aggregates Rg_1: and Rg_2: fractal dimension D_f and number $N_{part/agg}$ of particles per aggregate could not be calculated as parameters could not be fitted.
DLS	CEA	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 172.9±9.2. PdI:0.427±0.025 • Ultra-pure water dispersion (inter vial study) Z-average (nm): 176.9, PdI: 0.425
	NRCWE	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 147.5±4.5. PdI: 0.244±0.017 • Ultra-pure water dispersion (inter vial study) Z-average (nm): 146.8±0.06, PdI: 0.229±0.015
	INRS	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 245.7±37.2. PdI: 0.299±0.024
	JRC	• miliQ water dispersion (intra vial study). Z-average (nm): peak 1: 133, peak 2: 221, PdI: 0.490 • culture media dispersion (intra vial study) Z-average (nm): peak : 94.5, PdI: 0.123 • PBS dispersion (intra vial study) Z-average (nm): peak: 170.3, PdI: 0.202
TEM	CODA-CERVA, IMC-BAS	High porosity nanostructured material which may be considered aggregetes of primary silicon dioxide particles. Median diamater (nm): 48±4 Feret min: 33.5 nm (median of 4889) Feret max: 53.2 nm (median of 4889). % of aggregates <100nm: 77.5 % Morphology of aggregates/agglomerates: Low sphericity, angular.
TEM-tomography	CODA-CERVA	 Aggregates of very complex morphology composed of a variable number of interconnected primary subunits.
AFM	CEA	Third dimension of the agglomerates/aggregates: median (of 593): 24.2 nm.
Water Solubility		
24-hour acellular *in vitro* incubation	NRCWE	The 24-hour dissolution ratio of NM-203 was measured in three different media: 0.05% BSA in water, Gambles solution and Caco 2 media. Both NM-203 and the Al impurities are partially soluble in all media but amounts vary considerably with medium, as does the

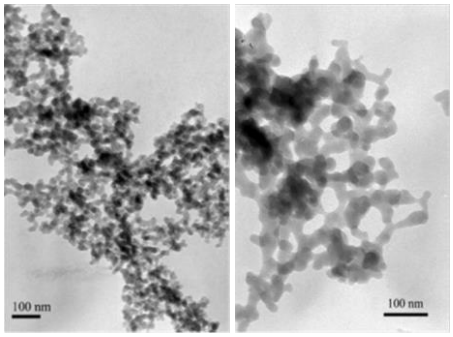
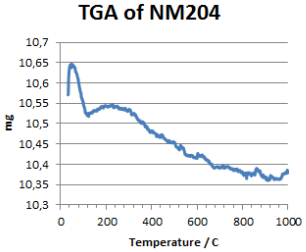
Method	Institution	Results for NM-203
test in special solutions		relative amounts of dissolved Al impurities compared with dissolved Si, suggesting that the solubility behaviour of the Al impurity and NM-203 depend on the medium.
Crystalline phase		
XRD	JRC	Synthetic amorphous silicon dioxide
	NRCWE	 Synthetic amorphous silicon dioxide.
	IMC-BAS	Synthetic amorphous silicon dioxide.
Dustiness		
Small Rotating Drum	NRCWE	Inhalable dustiness index (n=3) 5800±1488 Respirable dustiness index (n=3) 354±6
Vortex Shaker Method	INRS	Respirable dustiness index (n=1) 510000
Crystallite size		
SAXS	CEA	Parameters could not be fitted
XRD	JRC	Synthetic amorphous silicon dioxide
	NRCWE	Synthetic amorphous silicon dioxide.
	IMC-BAS	Synthetic amorphous silicon dioxide
Representative TEM picture(s)		
TEM	CODA-CERVA, IMC-BAS	 Aggregates with complex open structure.

Method	Institution	Results for NM-203
Particle size distribution		
SAXS	CEA	Parameters could not be fitted.
TEM	CODA-CERVA	Primary particle size: 13±6 nm
	IMC-BAS	Primary particle size: 45
	INRS	Primary particle size: 16±3 nm
TEM	CODA-CERVA, IMC-BAS	Number (expressed in %) of SAS NM particles smaller than 100nm, 50nm and 10nm <100 nm – 77.5%, <50 nm – 48.4% <10 nm – 0.3%
DLS	CEA	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. • Ultra-pure water dispersion (intra vial study) Z-average (nm): 172.9±9.2. PdI: 0.427±0.025, FWHM peak width: 82.5±11.3 • Ultra-pure water dispersion (inter vial study) Z-average (nm): 176.9, PdI: 0.425, FWHM peak width: 73.15
	JRC	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and revels the presence of large aggregates of few microns. • miliQ water dispersion (intra vial study). Z-average (nm): peak 1: 133, peak 2: 221, PdI: 0.490 • culture media dispersion (intra vial study) Z-average (nm): peak : 94.5, PdI: 0.123 • PBS dispersion (intra vial study) Z-average (nm): peak: 170.3, PdI: 0.202
	NRCWE	• Ultra-pure water dispersion (intra vial study) Z-average (nm): 147.5±4.5. PdI: 0.244±0.017, FWHM: 84.4±10.4 • Ultra-pure water dispersion (inter vial study) Z-average (nm): 146.8, PdI: 0.06, FWHM: 83.8±0.6
	INRS	The material is polydisperse. • Ultra-pure water dispersion (intra vial study) Z-average (nm): 245.7±37.2. PdI: 0.299±0.024
CLS	JRC	Peak (nm): 64, half width: 50, CLS Pdl: 1.35
Specific Surface Area		
BET	IMC-BAS	203.92 (m^2/g)
SAXS	CEA	167.2±13.4 (m^2/g)
TEM-tomography	CODA-CERVA	219±23 (m^2/cm^3) (Volume specific surface area)
BET	JRC	Sample stored at 40°C: single point: 192.4628 (m^2/g); multi point: 198.0809 (m^2/g). Sample stored at -80°C: single point: 189.8376 (m^2/g); multi point: 195.4241 (m^2/g).
Zeta Potential (surface charge)		
Zetametry	CEA	NM-203 forms a stable suspension, with negatively to neutral charged particles. The zeta potential, however, varied greatly as function of pH and reached -35 mV around pH 7. IEP 2-4
	JRC	Zeta potential at pH 6.6, milliQ water: -46.1 (mV). Zeta potential at pH 7.1, PBS: -18 (mV)

Method	Institution	Results for NM-203
Surface Chemistry		
XPS	JRC	The following elements were identified in the surface of NM-203: O (71.7 at%), Si (26.0 at%) and C (2.31 at%). The presence of C is considered to be due to surface contamination from hydrocarbons from air that have attached themselves to the material surface.
TGA	NRCWE	 No mass loss detec-ted. Phase transtion detected at 324 ºC (DTA). Observed mass-increase in TGA graph is due to boyancy.
Photo-catalytic activity		
End-point not relevant for SAS		
Pour-density		
Weighing	INRS	0.03 g/cm^3 (1 wt.% water content)
Porosity		
BET	IMC-BAS	Micropore volume (mL/g): 0.0
Octanol-water partition coefficient		
End-point not relevant		
Redox potential		
OxoDish fluorescent sensor plate for O_2 detection	NRCWE	The evolution of O_2 level during 24-hour incubation was measured in three different media. Different dO_2 values were observed for all applied media however in all three media the level of dO_2 increases with increased concentration of NM-203. The most profound increases with up to ca. 30 µmol O_2/l were observed in the 0.05% BSA water and Caco2 medium. The results suggest oxidative reactivity of NM-203.
Radical formation		
HPLC + UV	NRCWE	Using the benzoic acid probe to form 4 hydroxy benzoic acid in a phosphate buffered hydrous solution, gave no detectable concentration OH radicals.
Composition		
ICP-OES	CODA-CERVA	00.5-0.01%: Na in one of the vials tested
EDS	IMC-BAS	Al: 4300 ppm, S: 400 ppm, Si: 46.32 (wt %), O (wt%) calculated: 53.21

NM-204, summary of physical-chemical characterisation results

Method	Institution	Results for NM-204
Homogeneity		
DLS		Study not performed
Agglomeration / aggregation		
SAXS	CEA	Data regarding structure and size parameters not extracted from SAXS data.
DLS		Study not performed
TEM	CODA-CERVA, IMC-BAS	The amount of particles smaller than 100 nm is 71.2%. Quantitative study for aggregates/agglomerates not performed.
AFM		Study not performed
Water Solubility		
24-hour acellular *in vitro* incubation test in special solutions	NRCWE	The 24-hour dissolution ratio of NM-204 was measured in three different media: 0.05% BSA in water, Gambles solution and Caco 2 media. Both NM-204 and the Al impurities are partially soluble in 0.05% BSA in water and Caco2 media but amounts vary considerably with medium. In Gambles solution only NM-204 is partially soluble. The relative amounts of dissolved Al impurities compared with dissolved Si differ depending on medium, which suggests different solubility behaviour of the Al impurity and NM-204 depending on the medium.
Crystalline phase		
XRD	JRC	Synthetic amorphous silicon dioxide
	NRCWE	 Synthetic amorphous silicon dioxide
	IMC-BAS	Synthetic amorphous silicon dioxide
Dustiness		
Small Rotating Drum	NRCWE	Inhalable dustiness index (n=3) 24969±601 Respirable dustiness index (n=3) 1058
Vortex Shaker Method	INRS	Respirable dustiness index (n=1) 140000
Crystallite size		
SAXS	CEA	Amorphous material
XRD	JRC	Synthetic amorphous silicon dioxide
	NRCWE	Synthetic amorphous silicon dioxide
	IMC-BAS	Synthetic amorphous silicon dioxide

Method	Institution	Results for NM-204
Representative TEM picture(s)		
TEM	CODA-CERVA, IMC-BAS	 Aggregates with complex, open structure.
Particle size distribution		
SAXS	CEA	Equivalent diameter for spheres: 21nm (Primary particle size)
TEM	CODA-CERVA	Primary particle size: 10-15 nm (manual measurements)
	IMC-BAS	Primary particle size: 19
TEM	CODA-CERVA, IMC-BAS	Number (expressed in %) of SAS NM particles smaller than 100nm, 50nm and 10nm <100 nm - 71.2%, <50 nm - 36.4% <10 nm - 0.3%
DLS		Study not performed
CLS	JRC	Peak (nm): 98, half width: 203, CLS Pdl: 2.99
Specific Surface Area		
BET	IMC-BAS	136.6 (m^2/g)
SAXS	CEA	131±22.9 (m^2/g)
BET	JRC	Sample stored at 40°C: single point: 131.7462 (m^2/g); multi point: 134.3128 (m^2/g). Sample stored at -80°C: single point: 132.057 (m^2/g); multi point: 134.6187 (m^2/g).
Zeta Potential (surface charge)		
Zetametry		Study not performed
Surface Chemistry		
XPS	JRC	The following elements were identified in the surface of NM-204: O (71.9 at%), Si (23.2 at%), Na (0.5 at%) and C (4.3 at%). The presence of C is considered to be due to surface contamination from hydrocarbons from air that have attached themselves to the material surface.
TGA	NRCWE	Significant mass loss below 100°C (water). A 0.5 wt% gradual mass-loss above 110°C indicating e.g. loss of water associated in the micro pores or is associated with the presence of Na_2SO_4. 
GC-MS	NRCWE	GC-MS analysis results (retention time in min.): Tetramtehyl silicate: 4.9; Hexa-decanoic acid methyl ester: 33.4; Hexadecanoic acid: 33.9; Octadecanoic acid: 35.8
Photo-catalytic activity		
End-point not relevant for SAS		

Method	Institution	Results for NM-204
Pour-density		
Weighing	INRS	0.16 g/cm^3 (6 wt.% water content)
Porosity		
BET	IMC-BAS	Micropore volume (mL/g): 0.00666
Octanol-water partition coefficient		
End-point not relevant		
Redox potential		
OxoDish fluorescent sensor plate for O_2 detection	NRCWE	The evolution of O_2 level during 24-hour incubation was measured in three different media. Different dO_2 values were observed for all applied media however in 0.05% BSA in water and Gambles solution, for all three different concentration of NM-204, the same behaviour of dO_2 was observed. In case of Caco2 media a clear increment in dO_2 level was only observed at lowest dose suggesting O_2.The maximum O_2 changes observed for NM-204 is on the order of 30 µmol/ml. The results suggest that NM-204 generally has negligible redox activty.

APPENDIX VI: PROTOCOLS FOR CHARACTERISATION OF THE NANOMATERIAL SYNTHESISED IN KOREA

Information on Particle size distribution – dry and in relevant media

Methods

Media: Distilled water

Method/guideline followed: TEM and DLS size measurement procedures are as follows:

a) Protocol for TEM measurements

ⅰ) Sample preparation

- Holey carbon-coated copper TEM grids were chosen for the preparation of TEM nanoparticle specimens.
- One or two droplets of SiO_2 nanoparticle suspension were dropped onto the shiny side of the grids.
- The grids were dried in a desiccator.

ⅱ) Observation and analysis by TEM

- A 300 kV accelerating voltage was used (FEI Tecnai G2 F30 field emission gun (FEG) TEM).
- The microscope magnification was calibrated by imaging silicon {111} lattice fringes at the eucentric specimen position and assuming a 0.31355 nm spacing.
- Coarse focusing was accomplished by raising and lowering the specimen along the *z*-axis (optic axis) and fine focusing was accomplished by adjusting the objective lens current.
- As the actual magnification is sensitive to the objective lens current, the current was precisely controlled during experiments.
- Bright field images were collected using a CCD camera (UIltraScan^{TM}) and DigitalMicrograph software (Gatan, Inc.).
- The SiO_2 nanoparticle sizes were measured manually from the TEM images using DigitalMicrograph software (Gatan, Inc.).

b) Protocol for DLS measurement of a SiO_2 suspension using ELS-Z

- Measure the UV/Vis absorption spectrum of a 150 μL aliquot of the suspension between 200 and 800 nm against solvents (DW) as a baseline using a spectrophotometer (UV-1700, Shimadzu).
- Measure the DLS size on a 1 mL aliquot of the suspension using a particle analyzer (ELS-Z, Otzuka Electronics Co. LTD). Perform 15 runs/measurement × 5 measurements and average of 5 data points.
- Dilute the suspension in DW to 1/10 of the original concentration.
- Measure the DLS size of a 1 mL aliquot of the suspension.
- Repeat the dilution and DLS size measurement until the DLS size is constant within 20 %.
- Obtain the DLS size of SiO_2 nanoparticles from the most dilute suspension in the valid concentration range[9].
- *The instrument performance was qualified using a nanoparticle size reference (Gold Nanoparticle, RM 1980 – NIST).*

c) The protocol for the DLS measurement of a SiO_2 suspension using a Brookhaven particle size analyzer is described in "Development of Nano-materials Safety and Characterization Techniques" published by the Ministry of Education, Science and Technology, Korea, 2010. (KRISS).

[9] Valid concentration range means that the DLS size is constant within 20 %.

Year (study performed): 2010 - 2011

GLP: No

Analytical monitoring: TEM (FEI Tecnai G2 F30) and two Particle size analyzers (Brookhaven Instrument Co., ELS-Z, Otzuka Electronics Co. LTD. Japan)

Exposure period (duration): 1 year

Doses/concentration levels: 20 mg/mL for 30 nm SiO_2 nanoparticle suspension and

44 mg/mL for 40 nm SiO_2 nanoparticle suspension

Test conditions

Dilution water source: Distilled water

Stock and test solutions and their preparation: A stock suspension (> 150 mL) was prepared by mixing three batches (>50 mL) of synthesised SiO_2 nanoparticle suspensions. The suspension was stirred for 24 h using a magnetic bar. Three 1 mL aliquots of the suspension were sampled to obtain nanoparticle concentrations by drying-and-weighing. Next, 5 mL aliquots of the suspension were transferred to Teflon-capped vials and labelled with the time of characterization (0, 7, 14, 30, 60, 90, 180, 270 and 360 days after sampling). Each bottle was analyzed at the predetermined storage times.

Stability of the test chemical solutions: Suspensions were stable up to 12 months

Exposure vessel type: not described

Test temperature range: 26 - 28 °C (ambient temperature).

ZETA POTENTIAL/SURFACE CHARGE

Methods

Media: Distilled water

Method/guideline followed: The procedure for zeta potential measurement was as follows (*3 batches of SiO_2 nanoparticle suspensions were measured independently*):

- Measure the UV/Vis absorption spectrum of a 150 μL aliquot of the suspension between 200 and 800 nm against solvents (DW or pH-buffered solution) as a baseline using a spectrophotometer (UV-1700, Shimadzu).
- Measure the DLS size of a 1 mL aliquot of the suspension using a particle analyzer (ELS-Z, Otzuka Electronics Co. LTD.). Perform 15 runs/measurement × 5 measurements and average 5 independent data points.
- *The instrument performance was qualified using a nanoparticle size reference (Gold Nanoparticle, RM 1980 – NIST).*
- Measure the zeta potential of a 1 mL aliquot of the suspension using an electrophoretic mobility analyzer (Zetasizer Nano Z, Malvern). Perform 20 runs/measurement × 5 measurements and average 5 independent data points.
- *The instrument performance was qualified using a vendor-supplied -50 mV transfer standard referred to SRM 1980.*

The measurement procedure for suspension pH was as follows:

- Divide a 6 mL aliquot of the suspension into 4 aliquots of 1.5 mL each and transfer them into 2 mL microcentrifuge tubes. Centrifuge the solutions at 10,000 rpm for 20 min (HM-150IV, Hanil Science Ind.).

- Add 1.3 mL aliquots of the supernatant from the 4 microcentrifuge tubes to a 50 mL conical tube (Cat. No. 50050, SPL Lifesciences).
- Measure the pH of the DW and the supernatant using a pH meter (Orion 3 star pH Benchtop, Thermo Electron Co.) according to the following procedure. After each measurement, wash the pH electrode with DW.
  - Measure the solution pH (DW or supernatant) in a 50 mL conical tube (3 times).
  - Measure the pH of a pH 4.01 standard buffer solution (Orion 910104) (3 times).
  - Measure the solution pH (DW or supernatant) in a 50 mL conical tube (3 times).
  - Measure the pH of a pH 10.01 standard buffer solution (Orion 910110) (3 times).
  - Measure the solution pH (DW or supernatant) in a 50 mL conical tube (3 times).
- *The instrument performance was qualified using standard pH buffers of pH 4.01 (Orion 910104), pH 10.01(Orion 910110) and pH 7.00 (Orion 910107).*

Year (study performed): 2010-2011

GLP: No

Analytical monitoring: Zetasizer (Nano Z, Malvern, UK)

Exposure period (duration): 1 year

Doses/concentration levels: 20 mg/mL for 30 nm SiO_2 nanoparticle suspension and
44 mg/mL for 40 nm SiO_2 nanoparticle suspension

Test conditions

Dilution water source: Distilled water

Stock and test solutions and how they are prepared: A stock suspension (> 150 mL) was prepared by mixing three batches (>50 mL) of synthesised SiO_2 nanoparticle suspensions. The suspension was stirred for 24 h using a magnetic bar. Three 1 mL aliquots of the suspension were sampled to obtain nanoparticle concentrations by drying-and-weighing. Next, 5 mL aliquots of the suspension were transferred to Teflon-capped vials and labelled with the time of characterization (0, 7, 14, 30, 60, 90, 180, 270 and 360 days after sampling). Each bottle was analyzed at the predetermined storage times.

Stability of the test chemical solutions: Zeta potential was stable up to 3 months

Exposure vessel type: not described

Test temperature range: not described

APPENDIX VII: LIST OF ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
ANOVA	Analysis of Variance
APS	Aerodynamic Particle Sizer
ASASP	Association of Synthetic Amorphous Silica Producers
BET	Brunauer, Emmet and Teller
BSA	Bovine Serum Albumin
CEA	Commissariat à l'énergie atomique et aux énergies alternatives
CEN	Comité Européen de Normalisation
CLS	Centrifugal Liquid Sedimentation
CODA-CERVA	Veterinary and Agrochemical Research Centre (Belgium)
CPC	Condensation Particle Counter
DLS	Dynamic Light Scattering
ELPI	Electrical Low Pressure Impactor
EM	Electron microscopy
EDX	Energy-Dispersive X-ray spectroscopy
Fh-IME	Fraunhofer Institute for Molecular Biology and Applied Ecology, Germany
FMPS	Fast Mobility Particle Sizer
FWHM	Full-Width Half-Maximum
GLP	Good Laboratory Practice
h	hours
HEPA filter	High-Efficiency Particulate Air filter
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectrometry
IEP	Iso-Electric Point
IHCP	Institute for Health and Consumer Protection (JRC)
IMC-BAS	Institute of Mineralogy and Crystallography, Bulgaria
INRS	Institut National de Recherche et de Sécurite
ISO	International Organisation for Standardization
ISO/TC 229	ISO/Technical Committee on Nanotechnologies
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre, European Commission
L or l	Litre
LNE	Laboratoire national de métrologie et d'essais, France
lpm	Litre per minute
ml / mL	Milli litre
MWCNT	Multi Walled Carbon Nano Tube
NIST	USA National Institute of Standards and Technology
NM	Nanomaterial
NRCWE	National Research Centre for the Working Environment

OECD	Organisation for Economic Co-operation and Development
PSD	Particle Size Distribution
PBS	Phosphate Buffered Saline
PCS	Photon Correlation Spectroscopy
PdI	Poly Dispersion Index
pH	Acidity value
REACH	EU Legislation on Registration, Evaluation, Authorisation and restriction of Chemicals
RH	Relative Humidity
RMN	Representative Manufactured Nanomaterial
rpm	Rounds Per Minute
RSD	Relative Standard Deviation
RTM	Representative Test Material
s	second
SAS	Synthetic Amorphous Silicon Dioxide
SAS NMs	All of NM-200, NM-201, NM-202, NM-203 and NM-204
SAXS	Small Angle X-ray Scattering
SD	Standard Deviation
SD	Small Rotating Drum
SDR	Sensor Disk Reader
SEM	Scanning Electron Microscopy
SEM-EDS	Scanning Electron Microscopy-Energy Dispersive Spectroscopy
SIRT	Simultaneous Iterative Reconstruction Technique
SOP	Standard Operating Procedure
SCENIHR	Scientific Committee for Emerging and Newly Identified Health Risks
TEM	Transmission Electron Microscopy
USA	United States of America
USA-EPA	USA Environmental Protection Agency
USAXS	Ultra Small Angle X-ray Scattering
VS	Vortex Shaker
WPMN	Working Party on Manufactured Nanomaterials
w/w%	weight percent
XPS	X-ray Photoelectron Spectrometry
XRD	X-ray Diffraction