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DOSSIER ON SILICON DIOXIDE (JP AIST data on SiO2 UFP-80 and NanoTek) - PART 6 -

Series on the Safety of Manufactured Nanomaterials
No. 51

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Series on the Safety of Manufactured Nanomaterials

No. 51

DOSSIER ON SILICON DIOXIDE

(JP AIST data on SiO2 UFP-80 and NanoTek)

- PART 6 -

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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

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

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

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

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

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

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

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

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

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

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

ABOUT THE OECD

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

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

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

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PREAMBLE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FOREWORD

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

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

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

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

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

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

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

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

ACKNOWLEDGMENTS

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

TABLE OF CONTENTS

Name Other / JP AIST data on SiO_2 UFP-80 and NanoTek

Substance: Other / JP AIST data on SiO2 UFP-80 and NanoTek

1. GENERAL INFORMATION

1.1 Identification

Substance identification

Chemical name Other / JP AIST data on SiO_2 UFP-80 and NanoTek

1.2 Composition

1.3 Identifiers

1.4 Analytical information

1.5 Joint submission

1.6 Sponsors

1.7 Suppliers

1.8 Recipients

1.9 Product and process oriented research and development

2. CLASSIFICATION AND LABELLING

2.1 GHS

2.2 DSD - DPD

3. MANUFACTURE, USE AND EXPOSURE

3.1 Technological process

3.2 Estimated quantities

3.3 Form in the supply chain

3.4 Identified uses and exposure scenarios

3.5 Uses advised against

3.6 Waste from production and use

7.13 In vitro toxicological information

Endpoint study record: JP-AIST / Cellular influence including cytotoxicity

Administrative Data

Data source

Reference

Reference type	study report		
Author	Masanori HORIE	**Year**	
Title	"Characterisation Tests of Nanoparticles Using Human Cells" (in Japanese)		
Bibliographic source			
Testing laboratory	AIST	**Report no.**	KTN-09127
Owner company			
Company study no.		**Report date**	

Data access

data submitter is data owner

Materials and methods

Type of information

An in vitro cytotoxicity of SiO_2 nanoparticle was evaluated using 10 kinds of cultured cells. Particularly, cellular influences of human lung carcinoma A549 cells and human keratinocyte HaCaT were closely examined. The SiO_2 medium dispersion was well characterized.

Principles of method if other than guideline

An in vitro cytotoxicity study
To exclude artificial effects, protein adsorption ability of SiO_2 nanoparticles was prevented by treatment with bovine serum albumin. And stable SiO_2 nanoparticles-medium dispersion was prepared.SiO_2 nanoparticles were dispersed in 10% FBS supplemented DMEM. Cell viability, apoptosis, colony forming ability, intracellular ROS level (induction of oxidative stress) and DNA damage were evaluated.

Describe the scientific and technical basis of the test method

What biological/cellular model is the method based on?

Human keratinocyte HaCaT cells; Human lung carcinoma A549 cells; Human lung cancer LU99 cells; Human bronchioalveolar carcinoma NCI-H358 cells; Human lung fibroblast (normal) WI-38 cells; Human hepatocellular carcinoma Hep G2 cells; Human adenocarcinoma Caco-2 cells; Human pancreatic cancer KP-3 cells; Human adenocarcinoma HeLa cells; and Human monocytic leukemia THP-1 cells.

What biological endpoints/responses does this method address?

Cellular influence including cytotoxicity

What specific mechanisms associated with the biological response are targeted?

Oxidative stress and cell death (including apoptosis)

What methods/techniques are used for endpoints/responses determination?

Cell viability: Methyl thiazol tetrazolium (MTT) salt reduction assay, LDH assayColony forming ability: clonogenic assayOxidative stress: Intracellular ROS level: 2', 7'-Dichlorodihydrofluorescin (DCFH) method, Intracellular lipid peroxidation: diphenyl-1-pyrenylphosphine (DPPP) assay Apoptosis: Caspase-3 activityDNA damage: comet assayCells were exposed by SiO2 nanoparticles at concentration of 70-200 μg/ml.Exposure time: 6 and 24 h.

Are there potential technical limitations of this method for testing nanomaterials?

Yes. These methods are not suitable for evaluation of long term toxicity (e.g. carcinogenesis).

Describe the role of the method in context of hazard assessment for human health

What are specific limitations of this test in terms of predicting hazard to human health?

Relationship of the cellular exposure dose (in vitro) and the NOAEL (in vivo) is unknown.

Performance assessment of the method

Test materials

Details on test material

Denki Kagaku Kogyo, SiO_2 UFP-80 (Primary particle size: 34 nm, purity: >99.5%)
CIK NanoTek Corporation, SiO_2 NanoTek(R) (Primary particle size: 25 nm, purity: 99.9%)

Details on vehicle/medial matrix

Fetal bovine serum (FBS; CELLect GOLD; MP Biomedicals Inc.)Dulbecco's modified Eagle medium (DMEM; Gibco, Invitrogen, Life technologies)

Sample preparation/conditioning protocol

A stable dispersion was prepared by pre-adsorption and centrifugation. To prevent the starvation of cells because of the adsorption of medium components onto the surface of SiO_2 particles, SiO_2 particles were dispersed in FBS at a concentration of 80 mg/ml (approximately 1 M). Subsequently, the dispersion was centrifuged at 16,000g for 20 min. Precipitated SiO_2 nanoparticles were washed with FBS-free DMEM once and re-dispersed in an equivalent volume of fresh DMEM–FBS. The dispersion of nanoparticles in 10% FBS supplemented DMEM (DMEM-FBS) was centrifuged at 8000g for 20 min. After discarding the supernatant, the precipitate was re-dispersed in an equal volume of fresh DMEM–FBS. The resulting SiO_2 dispersion was again centrifuged at 4000g for 20 min. The above process was repeated until the supernatant collected was a stable SiO_2 dispersion. However, the centrifugal force was reduced gradually from 2000g to 1000g. The 4000g and the 1000g fractions of ultrafine SiO_2 were used for cellular examinations.

Method

Any other information on materials and methods incl. tables

Characterization of SiO_2-medium dispersionsIn these examinations, "secondary particle" and "average particle size" were defined as follows. A "secondary particle" is defined as a complex aggregate of primary particles, proteins from FBS and other medium components. In addition, "average particle size" is the size of the secondary particles as estimated from light-intensity measurements, under the assumption that the aggregate is globular. The Si SiO_2–DMEM–FBS dispersion prepared via the aforementioned method was divided into three parts, and they were used to perform simultaneous biological examinations and to take SiO_2-concentration and particle-size measurements. The secondary particle size in the SiO_2–DMEM–FBS dispersion was measured by dynamic light scattering (DLS). Details of this experiment are described elsewhere (Kato et al., Toxicol In Vitro. 23: 927-934, 2009).SiO2

concentration was measured by X-ray fluorescence (XRF) analyses. Briefly, 13 ml of metal oxide-DMEM-FBS dispersion was added to 13 ml of a standard solution (including 0.1 mg/ml of Fe as an internal standard element) and mixed well. Then, 5ml of the mixture was dried in an oven at 200 degrees C for 24 h. A dried sample was ground in an agate mortar. XRF was done using a dispersive X-ray fluorescence spectrometer. The amount of silicon was estimated from the molar ratio of silicon and the internal standard.

Results and discussions

Remarks on results including tables and figures

After 24-h exposure, SiO_2 nanoparticles did not influence to cell viability and cell membrane damage. The intracellular ROS level did not increase by SiO_2 exposure. Additionally, caspase-3 activity did not increase. DNA damage by SiO_2 exposure could not be observed. In some cell lines, colony formation was inhibited at high concentration of $SiO_2$2.

Applicant's summary and conclusion

Conclusions

Cellular influences of SiO_2 nanoparticles are small.Influences of SiO_2 nanoparticles on cell viability and cell membrane damage are small. SiO_2nanoparticles did not cause DNA damage and not increase theintracellular ROS level.

Cross-reference to other study

Kato H, Fujita K, Horie M, Suzuki M, Nakamura A, Endoh S, Yoshida Y, Iwahashi H, Takahashi K, Kinugasa S. (2010) Dispersion characteristics of various metal oxide secondary nanoparticles in culture medium for in vitro toxicology assessment. Toxicol In Vitro. 24:1009-1018.

8. ANALYTICAL METHODS

9. RESIDUES IN FOOD AND FEEDINGSTUFFS

10. EFFECTIVENESS AGAINST TARGET ORGANISMS

11. GUIDANCE ON SAFE USE

12. LITERATURE SEARCH

13. ASSESSMENT REPORTS

14. INFORMATION REQUIREMENTS