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DOSSIER ON TITANIUM DIOXIDE - PART 1 /3 - NM 105

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No. 54

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Series on the Safety of Manufactured Nanomaterials

No. 54

DOSSIER ON TITANIUM DIOXIDE - PART 1 /3 - NM 105

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

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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 format6 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 data contained within these dossiers is raw data and has not been evaluated by either the programme sponsors or the WPMN. Any conclusions found within these dossiers are under the responsibility of the researchers who made them. 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

[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)

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.

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 the Titanium Dioxide (TiO_2) manufactured nanomaterials which was prepared under the leadership of France and Germany. TiO2 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. The data is presented in an IUCLID[8] style format and includes the protocols and methods used (see Preamble).They are resulting from scientific literature and testing following harmonised guideline or protocols (like OECD Guidelines for the Testing of Chemicals)[9], or not

France and Germany led the Testing Programme on nano-TiO_2. This included the determination of data from the tests already completed using nano-TiO_2, a number of new tests from dedicated research project, as well as coordinating inputs provided and tests performed by other participating countries and stakeholder from Austria, Canada, Denmark, Spain, Japan, Korea, United Kingdom, United States, European Union, and the Business and Industry Advisory Committee to the OECD (BIAC).

Aeroxide®P 25 (P25) was chosen as ***principle material*** meaning that all the relevant endpoints have been addressed for this material.

- Aeroxide®P 25
  - provided and delivered by Degussa/Evonik, Lot-Nr.: 4168112198
  - provided and delivered by EC/JRC, Lot-Nr.: 4168031098 (called NM105)
  - US-NIST used the certified material SRM 1898, which was synthesised by NIST with the same properties than P25

At the same time, it was recognised that the nano-TiO_2 placed on the market presents high variability in its composition. With this in mind, additional materials were selected for performing a selected number of endpoints that could allow some comparability. As a consequence this allowed testing a broad range of material's characteristics and covering a broader range of exposure scenarios to human and the environment. These materials were:

[8] IUCLID is a software program 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.

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

- PC105 (JRC no. NM102)
    - provided by Cristal Global[10] and delivered by EC/JRC, Lot-Nr.: 6292000312
- Hombikat UV 100 (Sachtleben) identified as NM-101 Titanium Dioxide
    - provided and delivered by EC/JRC, Lot-Nr.: 10780048
- UV TITAN M212 (Sachtleben) (JRC no. NM104)
    - provided and delivered by EC/JRC, Lot-Nr.:808001
- UV TITAN M262 (Sachtleben) (JRC no. NM103)
    - provided by EC/JRC, Lot-Nr.:933002
- Tiona AT-1 (non-nano reference) (JRC no. NM100)
    - provided by Cristal Global[11] and delivered EC/JRC, Lot-Nr.: 6111007957

The materials were delivered to the participating laboratories including: i) product information; ii) certification of analysis; iii) storage conditions; and iv) Safety Data Sheet.

Material provided by EC/Joint Research Centre was bought from the commercially available sources or provided by the manufacturer. To assure the traceability, the materials delivered by the EC/JRC were homogenised, sub-sampled and kept under inert atmosphere according to paragraph 42 of the Guidance Manual for Sponsors before the delivery to the participating laboratories.

Finally, a literature review on TiO_2 was performed to gather all the available information on the selected nanomaterials, even though it was not necessarily from the same batches.

Due to the large amount of information generated throughout the OECD Testing Programme on TiO_2, the Dossier has been split in 6 parts, as follows:

- **Part 1**: **NM 105** (P25)
- **Part 2**: **NM 100** (Tiona AT-1 (non-nano reference))
- **Part 3**: **NM 101** (Hombikat UV 100)
- **Part 4**: **NM 102** (PC105)
- **Part 5**: **NM 103** (UV TITAN M262 (Sachtleben))
- **Part 6**: **NM 104** (UV TITAN M212)

Each part includes Annexes.

[10] Cristal Global handed over its material to EC/JRC at a later stage of the test programme.

[11] Cristal Global handed over its material to EC/JRC at a later stage of the test programme.

In the following document, an overview of the testing results, within the TiO_2 OECD Testing Programme, are presented. Detailed information on results and tests performed can be found in the technical dossiers of the particular TiO_2 nanomaterials.

During the elaboration of the dossier and because of variation observed for the same test performed with the same NM for one specific endpoint, it becomes an evidence that for an hazard assessment a well-considered review of the data for each end point has to be performed including the appropriateness of the test performances, information on exposure as well as information on the state of the NM within the test. Consequently, the lack of information about the state of the nanomaterial during the test performance (e.g. degree of agglomeration, interaction with other substances, different media used) conducts to a realistic exposition unknown.

Data within the dossier was gained by review of the literature as well as national and international projects, in particular like the European joint action Nanogenotox[12], which has covered both some mammalian toxicology and physical-chemical characterisation endpoints of the dossier or projects of the environmental research plan of the German Federal Ministry of Environment, Nature Conservation, Building and Nuclear Safety.

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.

[12] Nanogenotox is a European joint action, managed by France with 11 participating European countries and 17 laboratories. The list of participants is in the annex. (www.nanogenotox.eu)

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The OECD Secretariat and the Working Party on Manufactured Nanomaterials is which to thank France and Germany for leading the Testing Programme for Titanium Dioxide. In particular, Nathalie Thieriet from the Agency for Food, Environmental and Occupational Health & Safety "ANSES" (France), and Kathrin Schwirn from the Federal Environmental Agency UBA (Germany) who coordinated and led the testing of Titanium Dioxide, and Frank Herzberg from German Federal Institute for Risk Assessment (BfR) who reviewed the litterature. We are also truly grateful to those delegations that had participated in the testing:

Austria:

Vienna University, Department of Environmental Geosciences

Graz University, Institute of Pharmaceutical Science

Canada:

McGill University, Department of Chemical Engineering

NRC-BRI, Applied Ecotoxicology Group

Trent University, Environmental & Resource Studies Dept.

Health Canada, Environmental Health Science and Research Bureau

Health Canada, Healthy Environments & Consumer Safety Branch

Wilfrid Laurier University, Institute for Water Science

University of Victoria, Department of Biochemistry and Microbiology

HydroQual Laboratoris

University of Alberta, Biological Sciences

Denmark:

Technical University of Denmark, Department of Environmental Engineering

France:

Nanogenotox Partner's

ANSES, French Agency for Food, Environmental and Occupational Health & Safety (France), The Toxicology of Contaminants Unit, The Environmental Inorganic Contaminants and Mineral , The Department of Information, Communication and Dialogue with Society , The European and International Affairs Unit

BfR, Federal Institute of Risk Assessment (Germany), The molecular toxicology unit of the Department of Safety of Consumer Products

CEA, French Atomic Energy Commission (France), The Materials Sciences Division, The Life Sciences Division

CODA-CERVA, Veterinary and Agrochemical Research Centre (Belgium), The Electron Microscopy unit

EC/JRC, Joint Research Centre, Institute for Health and Consumer Protection (IHCP)

Nanotechnology

FIOH, Finnish Institute of Occupational Health (Finland), The New Technologies and Risks laboratory

IMB BAS, Roumen Tsanev Institute of Molecular Biology Bulgarian Academy of Sciences (Bulgaria), The Medical and Biological Research Laboratory

IMC BAS, Institute of Mineralogy and Crystallography Bulgarian Academy of Sciences (Bulgaria), Central Laboratory of Mineralogy and Crystallography

INRS, The Medical and Biological Research Laboratory, Aerosol Metrology Laboratory and the Inorganic Analysis and Aerosol Characterization Laboratory , Carcinogenesis and Developmental Toxicology Laboratory, Pollutants and Health Department

INSA, National Health Institute Doutor Ricardo Jorge (Portugal), The Genetic Toxicology R&D Unit

IPH, Scientific Institute of Public Health (Belgium), The laboratory of toxicology

IPL, Insitut Pasteur of Lille (France), The Genetic Toxicology Laboratory

ISS, Istituto Superior di Sanita (Italy), The Food and Veterinary Toxicology Unit

LNE, Laboratoire National de metrelogie et d"Essais, Laboratoire National de metrelogie et d"Essais

NIOM, The Nofer Institute of Occupational Medicine (Poland), The Laboratory of Molecular Toxicology

NRCWE, National Research Centre for the Working Environment (Denmark), Nanotoxicology and Occupational Hygiene Group

RIVM, National Institute for Public Health and Environment (The Netherlands), The Laboratory for Health Protection Research

UAB, Universitat Autonoma de Barcelona (Spain), The Group of Mutagenesis

Germany:

Institute of Energy and Environmental Technology (IUTA)., Air Quality & Sustainable Nanotechnology

Fraunhofer Institute of Toxicology & Experimental Medicine, Inhalation Toxicology & Chemical Risk Assessment

Fraunhofer Institute for Molecular Biology and Applied Ecology

RWTH Aachen, Institute of Ecochemistry,Ecology, and Ecotoxicology

University Frankfurt Main, Institute for Ecology, Evolution and Diversity

Technical University Dresden, Institute of process engineering and environmental technology

Hamburg University of Applied Sciences

Federal Institute for Materials Research and Testing, Materials and Air Pollutants

Japan:

National Metrology Institute of Japan, Advanced Industrial Science and Technology (AIST)

Korea:

Dongduk Women's University, College of Pharmacy

Hanyang University, Laboratory of Nanoscale Characterisation & Environmental Chemistry

Korea Research Institute of Standards and Science, Korea Research Institute of Standards and Science Division of Industrial Metrology

Seoul National University, School of Chemical & Biological Engineering

Kyung Hee University, Department of Applied Chemistry

Korea University, School of Life Science & Biotechnology

Spain:

INIA, Departamento de Medio Ambiente

USA:

NIST, Nanoparticle Measurements & Standards

EPA, National Health and Environmental Effects Research

EPA, Ecology Division

FDA, National Center for Toxicological Research

Finally, we would also like to acknowledge the effort done by the EC/Joint Research Centre in providing the materials, homogenised, sub-sampled and kept them under inert atmosphere before they delivered them to participating laboratories; as well as TDMA in providing some of the materials to JRC.

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7. TOXICOLOGICAL INFORMATION

7.1 Toxicokinetics, metabolism and distribution

Summary of toxicokinetic data of the HH literature data status 03rd April 2014

Inhalation route

Reference	Material/ Size	Test Organism (Strain)/ Test System	Method	Exposure/ dose	Main findings
Inhalation route					
Ferin et al. 1992	a. P25 b. 250 nm (Fisher)	Rat (Fisher 344)	1. Inhalation 2. intratracheal instillation	1. ~23 mg/m^3, 6 h/d, 5d/wk for 12 wks 2. 75-1000 µg or 500 µg	Nanoparticles retained longer in lung ($t_{1/2}$ = 501 days vs. $t_{1/2}$ = 174 days) and translocated to interstitium at a higher rate than fine particles.
Bermudez et al. 2004	P25 (Mean MMAD: 1.4 µm)	Rat (CDF (F344)/CrlBR) mouse (B3C3F1/CrlBR) hamster (Lak: LVG (SYR) BR)	Inhalation	0.5, 2.0, 10 mg/m^3 6h/d, 5h /wk, 13 wks + 4,, 13, 26, 52 weeks p.e. (hamster: 46 weeks)	Alveolar clearance of nanoparticles strongly retarded at highest concentration in rats, less in mice and only marginally in hamsters during postexposure. Post-exposure retention half-times in rats at low, mid and high dose levels were 63, 132, and 395 days, respectively
van Ravenzwaay et al. 2009	a. P25 b. 200 nm (Kronos)	Rat (Wistar)	Short-term inhalation test	a. 100 mg/m^3 b. 250 mg/ m^3 6h/d, 5d	Particles only detected in lungs and mediastinal lymph nodes. No particles detected in liver, kidney, spleen and basal brain with olfactory bulb (detection limit for Ti 0.5 µg/tissue). 200 nm particles translocated more efficiently, possibly because of massive agglomeration of the nanomaterial.
Creutzenberg et al. 2012	a. P25 (hydrophilic, 21 nm; Evonik) b. T805 (hydrophobic, 21 nm; Evonik)	Rat (Wistar)	Intratracheal instillation	0.3 mg/rat (single dose)	Calculated lung burden was 1238 µg/lung (near overload) for P25 and 5760 µg/lung (massive overload) for Bayertitan. Agglomerate size was measured following exposure in the alveolar space and in BAL. Either particle type showed a tendency to increasingly agglomerate over time (1h-28d p.e.) and being internalized, predominantly by alveolar macrophages (AM). P25 agglomerates formed larger and more rapidly agglomerates than T805 after uptake in lungs. De-agglomeration and particle entry into epithelial cells played no significant role under exposure conditions applied.
Eydner et al. 2012	a. P25 (anatase/rutile, pps: 21 nm, MMAD: 0.7 µm) b. Bayertitan (rutile, pps: 0.3 µm,	Rat (Wistar)	Inhalation (OECD 412, GLP)	21 d (6h/d) nose-only a. 10 mg/m3 b. 45 mg/m3) Recovery time points: 3, 28, 90 d p.e.	No significant difference in particle deposition at near-overload inhalation conditions in the lung between fine and nanoparticles. The main compartment for particles (agglomerates in case of nanoparticles) in TEM analysis proved to be intracellular in AM, followed by pneumocyte I type

	MMAD: 1.1 µm)				cells. Particles were absent from mitochondria or nuclei. A relevant difference in translocation to connective tissue in the lung was not found. However, a single nanoparticle-laden granulocyte was found in the capillary, indicating focal translocation..

Dermal route

Reference	Material/ Size	Test Organism (Strain)/ Test System	Method	Exposure/ dose	Main findings
Dermal route					
Gontier et al. 2008	a. P25 b. Eurosolex T-2000 c. Anthelios® XL SPF60	Human Pig (domestic) Mouse (SCID)	Topical	2 mg/cm^2 of 5% formulation for 2 h	No penetration of either particle formulation and skin type beyond stratum corneum as evidenced by high resolution electron and ion microscopy
Wu et al. 2009	a. P25 b. 4, 10, 25,60 90 nm commercial particles varying in crystallinity and surface charge	Mouse (BALB/c, *nu/nu*) Pig (domestic)	Topical (semi-occluded) Franz cell	400 µg/cm^2 per day for 60 d (mouse) 30 d exposure (pig ear (5% formulations)	Detection (AAS/TEM) of nanoparticles in a variety of secondary organs in mice including brain (P25 only) implied penetration of viable skin layers and dermal absorption. TiO_2 nanoparticles were detected in the epidermis of porcine skin after 30 d of exposure *in vivo*, whereas diffusion *in vitro* through porcine skin was negative.
Sadrieh et al. 2010	a. P25 b. T-Lite SF (coated; Bayer) c. Tipaque CR-50 (~400 nm; Ishihara)	Minipig (Yucatan)	Topical (High resolution imaging analysis)	2 mg cream (~ 30 mg/g Ti) /cm^2, 4x per d, 5d/wk for 4 wks	No significant penetration through the intact normal epidermis at prolonged exposure as evidenced by inductively coupled plasma mass spectroscopy and electron microscopy-energy dispersive x-ray analysis.

Other route

Reference	Material/ Size	Test Organism (Strain)/ Test System	Method	Exposure/ dose	Main findings
Other route					
Fabian et al. 2008	P25	Rat (Wistar)	OECD 417 (Intravenous injection)	5mg/kg	Rapid clearance from blood and even 28 d p.e. detection in liver > spleen > lung > kidney but not in blood, plasma, brain or lymph node by ICP-AES.

7.1.1 Basic toxicokinetics

Endpoint study record: toxicokinetics of intravenously administered P25 by JP-AIST

Administrative Data

other: Research program "Development of innovative methodology for safety assessment of industrial nanomaterials" supported by the Ministry of Economy, Trade and Industry (METI) of Japan

Purpose flag key study ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Study period 2011/01/05-2012/02/28
Reliability 1 (reliable without restriction)
Rationale for reliability Peer Reviewed Publication

Data source

Reference

Reference type study report
Author Nobuko Danno
Year 2012
Title Final report on Toxicokinetics tests and TiO2 analysis following intravenous administration of TiO2 nanoparticles [In Japanese]
Testing laboratory Kamakura Techno-Science Inc.
Report no. KTN-11105
Company study no. KTN-11105
Reference type publication
Author Naohide shinohara et al.
Year 2013
Title Tissue distribution and clearance of intravenously administered titanium dioxide (TiO2) nanoparticles
Testing laboratory Kamakura Techno-Science Inc.

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Objective of study
distribution
excretion
toxicokinetics

Principles of method if other than guideline
TiO2 nanoparticle dispersion was intravenously administred. Then the distribution to organs were analyzed with ICP-FSMS.

Reference Material/Nanomaterial and Sample identification number
Identifier Reference Material/Nanomaterial
Identity P25 TiO2

Test material identity
Identifier Common name
Identity TiO2 nanoparticles

Radiolabelling
no

Test materials
Details on test material
The Aeroxide TiO2 P25 nanoparticle (Lot No. 4161070798; Nippon Aerosil, Japan). The shape of the particle is spherical, the primary particle size is 21 nm, the specific surface is 50 m2/g and the purity is >99.5%. Aeroxide TiO2 P25 nanoparticle consists of approximately 70% anatase and 30% rutile. No chemical modification, organic solvents or surfactant was used to prepare a stable TiO2 nanoparticle suspension.

Test animals
Species
rat

Strain
other: SPF Fischer 344/DuCrlCrlj

Sex
male

Details on test animals and environmental conditions
age, 12 weeks; body weight, 218-270 g

Administration / exposure
Route of administration
intravenous

Vehicle
other: 0.2% disodium phosphate solution (food additive grade, Wako Pure Chemical Industries, Ltd., Japan)

Duration and frequency of treatment / exposure
Single intravenous administration.

Doses / concentrations
1 mg-TiO2/kg-bodyWeight

No. of animals per sex per dose
Five

Control animals
yes, concurrent vehicle

Positive control
no positive controls

Statistics
The differences in Ti or TiO2 organ tissue concentrations between the administration and control groups were statistically analysed by Student's t-test or Welch's test after F-test using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Decay curve fitting for the burden in organ tissues was conducted with a least-squares approach, in which P (((estimated value) - (measurement value))/(measurement value))2 was minimised using Solver tool in Excel 2010.

Results and discussions

Preliminary studies
no preliminary studies

Pharmacokinetic studies
Transfer into organs
Test No. #1
Transfer type other: Transfer to liver, spleen, lung, kidney, heart, and brain were determined.
Observation other: At 6 h, 94%, 2.0%, 0.17%, 0.023%, 0.014%, and 0.026% of administered TiO2 was found in the liver, spleen, lung, kidney, heart, and blood, respectively. No translocation to the brain was confirmed at a lower detection limit.

Excretion
The Ti excretion rates in the urine of the TiO2-administered group (mean ± SD: 0.27 ± 0.13 ng/h) did not significantly differ from that of the control group (0.43 ± 0.29 ng/h) for 7 days after administration. From 27 to 30 days after administration, the Ti content in urine was below the detection limit in both groups. The Ti excretion rates in the faeces of the TiO2-administered group (15 ± 2.9 ng/h) also did not differ from that of the control group (18 ± 4.0 ng/h) over 7 days after administration. Since three rats and two rats did not produce faeces between 0 and 6 h after administration in control and administration groups, the average Ti content in faeces was small between 0 and 6 h after administration.

Toxicokinetic parameters
Test No. #1 AUC: 218 ng hr/ml

Metabolite characterisation studies

Metabolites identified
not measured

Applicant's summary and conclusion

Interpretation of results

bioaccumulation potential cannot be judged based on study results

Conclusions

In the present study, the tissue distribution, clearance, and excretion of TiO2 nanoparticles (P25) after intravenous administration were determined using a highly sensitive analytical method. We observed 94%, 2.0%, 0.17%, 0.023%, 0.014%, and 0.026% of administered TiO2 present in the liver, spleen, lung, kidney, heart, and blood at 6 h after the administration. The TiO2 burden in the liver and spleen did not change over 30 days, while that in the lung, kidney, and blood decreased with time; these decreases were statistically significant although they were minimal. Clearance from the lung and kidney was fitted with a 2-step decay model and 1-step decay model, respectively. The partition data obtained in the present study may be valuable to the future development of PBPK modeling of TiO2 nanoparticles.

Cross-reference to other study

Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI. (2004). Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci 77 (2): 347–357. van Ravenzwaay B, Landsiedel R, Fabian E, Burkhardt S, Strauss V, Ma-Hock L. (2009). Comparing fate and effects of three particles of different surface properties: Nano-TiO2, pigmentary TiO2 and quartz. Toxicol Lett 186:152–159. Wang JX, Zhou GQ, Chen CY, Yu HW, Wang TC, Ma YM, Jia G, Gao YX, Li B, Sun J, Li YF, Jiao F, Zhao YL, Chai ZF. (2007). Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett 168: 176–185. Ma-Hock L, Burkhardt S, Strauss V, Gamer A, Wiench K, van Ravenzwaay B, Landsiedel R. (2009). Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance. Inhalation Toxicology 21(2): 102–118. Fabian E, Landsiedel R, Ma-Hock L, Wiench K, Wohlleben W, Ravenzwaay B. (2008). Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats. Arch Toxicol 82:151–157 Kreyling WG, Wenk A, Behnke M. (2010). Quantitative biokinetic analysis of radioactively labelled, inhaled Titanium dioxide Nanoparticles in a rat model. Federal Environment Agency (Umweltbundesamt). http://www.umweltdaten.de/publikationen/fpdf-l/4022.pdf.

Endpoint study record by Fraunhofer ITEM

Administrative Data

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

Data source

Reference

Reference type study report
Author Otto Creutzenberg
Year 2013

Title Toxic Effects of Various Modifications of a Nanoparticle Following Inhalation
Bibliographic source BAuA Research Project F 2246
Testing laboratory Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) Nikolai Fuchs Strasse 1 30625 Hannover, Germany
Report no. Fraunhofer ITEM Study No. 02 N 11 538
Owner company Federal Institute for Occupational Safety and Health

Materials and methods

Type of method

in vivo

Test guideline
Qualifier according to
Guideline other guideline: OECD 412
Deviations yes 90 days post exposure

Test animals

Species
rat

Strain
Wistar

Sex
male

Details on test animals and environmental conditions
Male Wistar rats [strain Crl:WI (Han)] were purchased from Charles River Deutschland (Sulzfeld, Germany). The age of the animals at the start of exposure was approx. 8 weeks and the weight approx. 270 gram. Rats were exposed to the test item by nose-only inhalation. For a period of 2 - 3 weeks prior to exposure animals were trained to become accustomed to nose-only tubes.

Administration / exposure

Route of administration

inhalation: aerosol

Details on exposure
Rats were exposed to aerosol concentrations (low, mid, high) of 3, 12 and 48 mg/m3 for 28 days (6 hours/day, 5 days/week) while concurrent controls inhaled clean air. This dosing scheme was aiming at achieving non-overload, partial overload and complete overload conditions in the low, mid and high dose groups, respectively. Subsequently, endpoints were analysed at day 3, 45 and 94 of the post-exposure period. Calculation: According to the MPPD model the deposition rate for particles with an agglomerate density ρAgg = approx. 1.7 and an MMAD of approx. 1 µm amounts to approx. 7%. In a 28-day test the retained particles masses would result in approx. 0.20, 1.0 and 6 mg/lung, respectively.

Control animals
yes

Details on study design

All animals were subjected to a complete necropsy, which included a careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. For anesthesia an overdose of carbon dioxide was used. The abdominal cavity was opened and the diaphragm was cut carefully allowing the lungs to collapse. Heart, esophagus, upper half of trachea, thymus and lung associated lymph nodes (LALN) were removed from the lung convolution. The lung was inflated under a pressure of about 20 cm water with formalin and was fixed by immersion for a minimum of 2 hours, and used for histopathology. Thereafter the weight of the lower part of the trachea was recorded and the weight of the lung was calculated. The following organs were trimmed and wet weights were recorded: liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain, and heart. All tissues listed in OECD Guideline no. 412, table 2 were prepared for histopathology. The trimming was done according to Ruehl-Fehlert et al. (2003), Kittel et al. (2004) and Morawietz et al. (2004). The following histopathology was performed in 6 animals per group after end of exposure (day 3) and in the recovery group animals on day 45 and day 94 after exposure: · full histopathology on the respiratory tract and other organs and tissues, as listed in OECD 412 of all animals in the clean air control group and the high dose groups and all animals that died or were killed during the study. · histopathology of the left lung lobe, including bronchi and the lung-associated lymph nodes (LALN), trachea, larynx, pharynx, the nasal cavities (turbinales) and visceral pleura in all animals of all groups. Lungs were fixed in buffered formalin (10%), embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E). A special stain was applied for diagnosis of fibrotic changes: Masson trichrome. After sacrifice the right lung lobes were used for transmission electron microscopy (TEM) analysis. Transmission Electron Microscopy (TEM) Analysis: At the respective time points to be investigated (recovery day 3, 45 and 94) the right part of the lung including the right cranial, right middle and right caudal lung lobe as well as the accessory lung lobe were fixed by instillation using 5% glutaraldehyde solution (pH 7.2) for at least 24 hours. Following fixation of the tissue, the volume of the fixed tissue was determined using the method of Scherle (1970). The basic principal of this method is that the displacement of water equals the volume of the object. The displaced water leads to an increased weight which can be measured. The volume of the lung tissue was measured to detect a possible age- or treatment-dependent affect on the lung volume which would influence the measurement of the particle amount in the transmission electron microscope. Thereafter, to avoid biased sampling, multiple specimens per organ were taken using the systematic uniform random sampling method. These samples were postfixed in 1% osmiumtetroxid in 0.1M sodium cacodylate buffer. Following dehydration of the tissue in a graded series of ethanol the specimens were infiltrated with epoxy resin and embedded. An ultramicrotome (Ultracut E, Richard-Jung) was used for cutting ultrathin sections (70nm) of three randomly chosen samples per animal. They were positioned on copper grids and observed with a transmission electron microsope (Zeiss, Leo 910). 55000 square micrometer of each sample were investigated at a magnification of 10.000x. The amount and location of the nanoparticles found was noted and assigned to compartments. The compartments in which nanoparticles were detected were defined as follows: intraalveolar macrophage, free within the alveolus, pneumocyte type I, pneumocyte type II, free within the interstitium, interstitial macrophage, interstitial cellular (not otherwise definable), bronchiolar epithelium. For statistical analysis of the transmission electron microscopy results the software Statistica (Statsoft, USA) was used and an analysis of variance was applied.

Statistics

Differences between groups were considered statistically significant at $p < 0.05$. Data were analyzed using analysis of variance. If the group means differed significantly by the analysis of variance the means of the treated groups were compared with the means of the control groups using Dunnett's test. The statistical evaluation of the histopathological findings will be done with the twotailed Fisher test by the PROVANTIS system. If necessary, further statistical procedures will be applied upon agreement with the sponsor.

Overall remarks, attachments

Overall remarks

for results see attachment

Attached full study report

Attached document: 02N11358_300912_final_090413.pdf (general annex): ENV/JM/MONO(2015)17/ANN21

Endpoint study record: Basic toxicokinetics_NM 105_IV by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	W De Jong	**Year**	2013
Title	Deliverable 7: Identification of target organs and biodistribution including ADME parameters		
Bibliographic source			
Testing laboratory	IMB-BAS (BG)	**Report no.**	D7
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: Nanogenotox

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Test materials

Details on test material

Commercial name: Aeroxide P25 (Evonik)

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Administration / exposure

Route of administration

intravenous

Vehicle

other: Rat Serum Albumin (RSA) 0.05% diluted (9:1) v/v in 10 x phosphate buffer pH 7.4.

Duration and frequency of treatment / exposure

Administration: Single (day 1) or repeated (on 5 consecutive days, day 1-5) Sampling time: - Single admin: day 2 and day 90 - Repeated admin: day 6, 14, 30 and 90

Doses / concentrations

2.3 mg of TiO2 resulting in a dose of 8.7-9.7 mg/kg bw/d (male) and 12.4-13.7 mg/kg bw/d (female) 5 day cumulative dose: 43.5-48.5 mg/kg bw (male) and 62-68.5 mg/kg bw (female)

No. of animals per sex per dose

Treated Groups: 3-4 M + 2-3 F Control: vehicle 3-4 M + 2-3 F

Control animals

yes

Details on dosing and sampling

Sampling tissues: liver, spleen, kidneys, lung, brain, bone including bone marrow (femur)

Overall remarks, attachments

Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D7 Acute Tox: **ENV/JM/MONO(2015)17/ANN11** D7__Biodistribution: **ENV/JM/MONO(2015)17/ANN12**
Remarks	Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

other: Major target organs following single administration : liver > spleen >lung > kidney to a lesser extent Major target organs following repeated administration: liver > spleen> lung and kidney to a lesser extent. Significant accumulation in brain and heart c

Conclusions

NM 105 is rapidly distributed from the bloodstream to the organs with liver > spleen >lung > kidney to a lesser extentIts concentration declines between day 6 and 90 after administration in all the organs although level of Ti remains above the control liver and spleen.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Basic toxicokinetics_NM 105_Gavage by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	W De Jong	**Year**	2013
Title	Deliverable 7: Identification of target organs and biodistribution including ADME parameters		
Bibliographic source			
Testing laboratory	NRCWE (DK)	**Report no.**	D7
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Test materials

Details on test material

Commercial name: Aeroxide P25 (Evonik)

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: Rat Serum Albumin (RSA) 0.05% diluted (9:1) v/v in 10 x phosphate buffer pH 7.4.

Duration and frequency of treatment / exposure

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

Doses / concentrations

2.3 mg of TiO2 resulting in a dose of 10.2-11.4 mg/kg bw/d (male) and 13.1-15.2 mg/kg bw/d (female). 5 day cumulative dose: 51-57 mg/kg bw (male) and 65.5-76 mg/kg bw (female)

No. of animals per sex per dose

Treated Groups: 3 M + 3 FControl: vehicle 2 M + 3 F

Control animals

yes

Details on dosing and sampling

Sampling: Gastrointestinal tract, liver, spleen, , lungs, lymph nodes (mesenteric and popliteal),Body Fluids Sampled: Feces sampling: -Repeated admin (day 5): day 6

Overall remarks, attachments

Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D7 Acute Tox: **ENV/JM/MONO(2015)17/ANN11** D7__Biodistribution: **ENV/JM/MONO(2015)17/ANN12**
Remarks	Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

no bioaccumulation potential based on study results

Conclusions

No evidence for uptake of NM-105 following a 5 day exposure via oral route (gavage)

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

7.2 Acute Toxicity

7.2.1 Acute toxicity: oral

7.2.2 Acute toxicity: inhalation

Intratracheale installation

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
intracheal instillationn						
Driscoll and Maurer 1991	a. 0.02 µm (Degussa; P25?) b. 0.3 µm, Fisher Scientific)	rat	Intratracheal instillation	10 mg/kg	POD < 10 mg/kg	Elevated neutrophil numbers and fibrosis correlated with increased release of TNF-alpha and fibronectin in case of ultrafine TiO_2. In contrast to fine TiO_2, no IL-1 release which was in line with absence of granulamatous response.
Oberdörster et al. 1992	a. P25 b. 250 nm (Fisher)	rat	Intratracheal instillation	65, 107, 200, 500, 1000 µg	NO(A)EL ≈ 0.3 mg/kg	P25 showed similar PMN response at much lower mass levels compared to 250 nm particles. Increase in PMN numbers observed at the lowest P25 dose level tested. No correlation with other adverse effects was reported in this experiment.
Ferin et al. 1992	TiO_2 of various sizes (12, 21, 230, and 250 nm) including P25	rat	Intratracheal instillation	500 µg	POD < 2 mg/kg	Increased lung burden with decreasing size accompanied by a transient acute inflammatory response.
Osier et al. 1997	a. P25 b. 250 nm (Fisher)	rat	1. Intratracheal instillation 2. Intratracheal inhalation	1. 500 µg (a) and 750 µg (b) 2. 125 mg/m^3 for 2 h	POD < 2.5 mg/kg	P25 showed slightly higher acute inflammatory response as indicated by a number of BALF parameters and a major role of MIP-2 (chemotactic factor for PMN). No correlation with other adverse effects was reported. Instillation elicited a higher inflammatory response than inhalation.
Rehn et al. 2003	a. P25 b. T805 (20 nm, hydro-phobic)	rat	Intratracheal instillation	0.15, 0.3, 0.6, and 1.2 mg	NOAEL≈ 0.68 mg/kg	Low dose instillation with nano-TiO_2 resulted in transient pulmonary inflammation with increased PMN numbers in BALF being the most sensitive parameter.

Renwick et al. 2004	a. P25 (?) Tioxide)	rat	Intratracheal instillation	125 or 500 µg	POD ≈ 0.3 mg/kg	Elevated inflammatory BALF parameters only at high dose and more pronounced in case of P25 together with epithelial damage. An increased chemotactic behaviour of AM isolated from P25-treated rats.
Chen et al. 2006	P25 (?)	mouse	Intratracheal instillation	0.1 or 0.5 mg	POD ≈ 3.3. mg/kg	Induction of pulmonary emphysema, possibly caused by placenta growth factor PIGF and related inflammatory pathways.
Warheit et al. 2007a	a. P25 ("uf TiO_2") b. "uf-A" (136 nm, rutile) c. "uf-B" (150 nm, rutile) d. fine TiO_2 (380 nm, coated rutile)	rat	Intratracheal instillation	1 or 5 mg/kg	POD > 1 mg/kg (?)	P25 induced pulmonary inflammation, cytotoxicity cell proliferation and adverse lung effects (not specified further): Ranking of inflammatory response: P25 > fine-sized TiO_2 = uf-A = uf-B. Differences possibly due to crystal structure, inherent pH of the particles, or surface chemical reactivity.
Warheit et al. 2007b	a. P25 ("uf-3") b. "uf-1" (136 nm, rutile) c. "uf-2" (150 nm, rutile) d. "F1" (380 nm, coated rutile)	rat	Intratracheal instillation	1 or 5 mg/kg	POD > 1 mg/kg	Ranking of toxicity: uf-3 > F-1 = uf-1 = uf-2. P25 produced sustainable pulmonary inflammation, cytotoxicity and adverse lung tissue effects. Differences possibly due to crystal structure, inherent pH of the particles, or surface chemical reactivity.
Li et al. 2007	a. 3 nm (synthesized) b. 20 nm (P25?, Shanghai Huijing)	mouse	Intratracheal instillation	0.4, 4 and 40 mg/kg	NOAEL = 0.4 mg/kg	3 nm particles did not produce more pulmonary toxicity than the 20 nm particles at any dose level. The pH was identified as an important toxicity trigger.
Grassian et al. 2007a	a. 5 nm (anatase) b. P25 (?) (anatase/ rutile)	mouse	Intranasal instillation	a. 0.1, 0.4, 0.6 mg/ml b. 0.5, 2.0, 3.0 mg/ml	NOAEC = 0.1 mg/ml	Several BALF parameters and histopathology revealed that larger particles were only slightly more toxic.
Sager et al. 2008	a. P25 ("UFTiO_2) b. Fine TiO_2 ("FTiO_2") (rutile, 1 µm, Sigma)	rat	Intratracheal instillation	0.0313, 0.0625 and 0.125 cm^2/cm^2 (0.26, 0.52, 1.04 mg UF TiO_2 and		2P25 was at least 41 fold more potent than fine TiO2 on a mass dose basis with regard to pulmonary inflammation and lung damage. Normalization to surface area doses revealed the major role of surface area in bringing about

				5.35, 10.7 and 21.41 mg for $FTiO_2$)		toxicity.
Oberdörster 2001	a. P25 b. Fine TiO_2 (250 nm)	rat	Intratracheal Instillation (+ LPS priming)	50 µg Priming : 70 endotoxin units LPS	n.a.	P25 higher inflammatory response (PMN in BALF) compared to fine particles, both in the absence or presence (synergistic effect!) of LPS.
Ahn et al. 2005	0.29 µm (DuPont)	rat	Intratracheal Instillation	4 mg/kg	POD < 4 mg/kg	Induction of goblet hyperplasia, mucin gene expression, and increased IL-13 production in mast cells.
Park et al. (2009)	P25	mouse	Intratracheal Instillation	5, 20, 50 mg/kg	LOAEL < 5 mg/kg	Induction of pro-inflammatory cytokines in BALF and blood. Granuloma formation in lung tissue and other elevated pro-inflammatory parameters implied induction of chronic inflammation

Endpoint study record: Acute toxicity: inhalation.001 by JP-AIST

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Study period From 2010-11-11 To 2011-4-5
Reliability 2 (reliable with restrictions)
Rationale for reliability No guideline, but the study was desinged on the basis of scientific article (Kobayashi et al. Toxicology 264, 110-118, 2009) and communication with the authors.

Data source

Reference

Reference type study report
Author Kohei Mizuno
Year 2011
Title Intratracheal administration study of titanium oxide nanoparticles into rats
Testing laboratory DIMS Institute of Medical Science, Inc.
Report no. 1028
Owner company AIST Japan
Company study no. 1028
Report date 2011-04-12

Data access
data submitter is data owner

Data protection claimed
yes, but willing to share

Materials and methods

Test type
other: intratracheal instillation in rats

GLP compliance
no Animal husbandly, procedures, data handling, etc., were carried out in accordance with in-house SOPs.

Test materials

Reference Material/Nanomaterial and Sample identification number
Identifier Reference Material/Nanomaterial
Identity NM105; Aeroxide P25

Test material identity

Identifier IUPAC name
Identity titanium(IV) oxide

Details on test material
Aeroxide P25 (OECD principal material of TiO2 sponsorship program)

Test animals

Species
rat

Strain
other: Crl:CD(SD) SPF

Sex
male

Details on test animals and environmental conditions
TEST ANIMALS - Source: Charles River Laboratories Japan Inc. - Age at study initiation: 8 weeks - Weight at study initiation: 296-351 g - Fasting period before study: - Housing: rat cage (plastics, W257xL426xH200 mm) - Diet (e.g. ad libitum): solid pellet, ad libitum - Water (e.g. ad libitum): drink tap water, ad libitum - Acclimation period: 8 days ENVIRONMENTAL CONDITIONS - Temperature (°C): 20.0-24.0 - Humidity (%): 47-70 - Air changes (per hr): more than 10 times - Photoperiod (hrs dark / hrs light): 12 hours light IN-LIFE DATES: From: To:

Administration / exposure

Route of administration
other: liquid dispersion

Type of inhalation exposure
other: intratracheal instillation

Vehicle
other: 30 mM phosphate buffer solution + 2 mg/mL Tween 80

Details on inhalation exposure
Test solution was prepared by suspending the test substance in 30 mM phosphate buffer solution containing 2 mg/mL of Tween®80 (MP Biomedicals) in concentrations of 1 and 5 mg/mL for a higher and lower doses, respectively. The test solution was sonicated with ultrasonic bath (Elmasonic S30H, 37kHz, 280W) for 30 minutes to achieve homogeneous dispersion. For the instillation, a disposable syringe and a DIMS intratracheal administration tube (R-1) were connected using a Luer bulb. Under isoflurane (Escain®, Mylan Inc.) anesthesia, 1.0 mL/kg of the test solution was intratracheally administered to rats. Test solution was administered only once at the start of the experiment.

Concentrations
5 mg/kg

No. of animals per sex per dose
20
Control animals
yes

Details on study design
- Duration of observation period following administration: 13 weeks - Frequency of observations and weighing: twice a day for general status, and once a week for weighing. - Necropsy of survivors performed: yes - Other examinations performed: clinical signs, body weight,organ weights, histopathology, other: BALF analysis (cell fraction, LDH, and micro TP)

Statistics
The control groups and the test substance groups were compared to determine whether differences between them were statistically significant. Results were interpreted using a significance level of 5% ($P < 0.05$) or 1% ($P < 0.001$). F-tests were performed using the Bartlett method at a significance level of 5% on the mean body weight, mean organ weight, and mean values for the BALF parameters. If the variance was homogeneous, a two-tailed test using the parametric Dunnett s method was performed. If the variance was not homogeneous, a two-tailed test using the non-parametric Steel method was performed. F-tests were performed to compare body weight, organ weight, and the values of BALF parameters between Group Nos. 1 and 2. If homogeneity of variance was confirmed, a two-tailed Student's t test was performed. If homogeneity of variance was not confirmed, a two-tailed Welch test was performed. To compare differences in the frequency of macroscopic pathological findings or histopathological changes, each substance group was compared against the control group using Fisher' s exact probability test (one-tailed). Comparisons of the severity of graded lesions was performed using the Wilcoxon test (two-tailed). No statistical analysis was performed on observations of the general status.

Results and discussions

Mortality
No deaths occurred in any group throughout the duration of the study.

Clinical signs
Crepitus was noted in all animals following test substance administration, but it disappeared on the next day. Thereafter, no significant changes were observed.

Body weight
No statistically significant difference from the vechicle control group was observed.

Gross pathology
One to many white spots/areas were found in the lungs. However, no differences in the areas or numbers of these spots were determined among the test substances concurrently tested, and no relationship was found between the white spots/areas and other parameters.

Other findings
- Organ weights: Both the absolute and relative lung weights were significantly higher than in the vehicle control group at Week 1. - Histopathology: Findings in lung were scarce to moderately scarce appearance of macrophages in the alveolar space, localized scarce appearance of forming macrophage foam cells, scarce to moderately scarce infiltration of perivascular mononuclear and inflammatory cells, mild granulation, scarce appearance of intra-alveolar foreign bodies, mild bleeding, scarce hemoglobin crystals, and mild thickening of the alveolar and bronchiolar epithelia. Among these findings, the appearance of macrophages in the alveolar space was significant from Day 3 to Week 13, as compared with the vehicle control group. Moreover, perivascular inflammatory cell infiltration, appearance of intra-alveolar foreign bodies, and thickening of the bronchiolar epithelium were also significant at Day 3 and Week 1. Finally, thickening of the alveolar epithelium was significant at Day 3 and Week 13. At Week 13, however, the localized appearance of forming macrophage foam cells was significantly less frequent. - Other observations: At Day 3, significant increases in the WBC count, neutrophil count ratio, and eosinophil count ratio, and a significant decrease in the monocyte count ratio were observed as compared with the vehicle control group. At Week 1, significant increases in the WBC count, neutrophil count ratio, and lymphocyte count ratio, and a significant decrease in the monocyte count ratio were observed. At Weeks 4 and 13, no statistically significant differences were observed between the vehicle control group and the substance group.

Overall remarks, attachments

Attached full study report
Attached documents: acute_pulmonary (additional annex): ENV/JM/MONO(2015)17/ANN20

Applicant's summary and conclusion

Conclusions

Acute to subacute (3 months) pulmonary response was examined by intratracheal instillation study of single dose (5 mg/kg). WBC counts and biochemical markers contained in bronchoalveolar lavage fluids and histopathological examination showed inflammatory changes in lung from three days after the instillation. The inflammatory response was not considered strong because the severity of these reactions gradually decreased over time, and no granulation or fibril formation was observed in any rat.

Endpoint study record: Intratracheal instillation by by Fraunhofer ITEM

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Study period early 2013

Data source

Data access

other: performed and provided by Fh-ITEM

Cross-reference to same study

ITEM Study No. 02 N12 516

Materials and methods

Test type

other: Intratracheal instillation

Principles of method if other than guideline

Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, Schlesinger RB. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. Toxicol Sci. 2000 May; 55(1):24-35. Total dose given in two aliquots on two consecutive days, each suspended in 0.3 ml saline.

Test animals

Species

rat

Strain
Wistar

Sex
male

Administration / exposure

Details on inhalation exposure
According to D. Schaudien, J. W. Knebel, I. Mangelsdorf, J.-U. Voss, W. Koch, O. Creutzenberg "Dispersion and Retention of Dusts Consisting of Ultrafine Primary Particles in Lungs" but using ultrasound with higher dose instead of UltraTurrax. total dose: 1,5mg/lung

Concentrations

4-wk Intratracheal Instillation Study with subsequent bronchoalveolar lavage (BAL) on days 3 and 27 1.5mg/rat Administration of total dose in two aliquots on consecutive days (day -2, day -1)

No. of animals per sex per dose

5 ->day 3 5 ->day 27

Control animals

yes

Results and discussions

Preliminary study (if fixed dose study)
see attached document

Overall remarks, attachments

Attached background material
Attached document 17: OECD_TiO2_IT-Test_BAL_020513.pdf: ENV/JM/MONO(2015)17/ANN14

Applicant's summary and conclusion

Conclusions

Ranking of toxic potential based on the results of this intratracheal instillation test: Day 3: NM-105 = NM-104 > NM-103 >> Hombikat > NM-101 = PC105 > TIONA AT-1 = vehicle control Ranking of toxic potential based on the results of this intratracheal instillation test: Day 27: NM-105 > NM-104 > NM-103 >> Hombikat > NM-101 = PC105 = TIONA AT-1 = vehicle control Full recovery for Hombikat UV 100, NM-101, PC105 and TIONA AT-1 after 27 days

7.2.3 Acute toxicity: dermal

7.2.4 Acute toxicity: other routes

Summary of the HH literature data of acute toxicity data (inhalation route status 03rd April 2014 Summary)

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
Other routes						
Fabian et al. 2008	P25	rat	- Intravenous injection	5 mg/kg	LOAEL > 5mg/kg	No obvious toxic or adverse health effects. No detectable inflammation or organ toxicity up to 28 d p.e., based on 67 different biomarkers in blood
Moon et al. 2010	P25	mouse	Intraperitoneal injection (± LPS priming)	40 mg/kg (Priming: 5mg/kg LPS, i.p.)	LOAEL = 40 mg/kg	P25 induced acute pulmonary inflammatory effects: Increased influx of neutrophils and ROS activity; elevation of proinflammatory cytokines and activation of NF-kappaB pathway. P25 acted synergistically with LPS.

7.3 Irritation / corrosion

7.3.1 Skin irritation / corrosion

Endpoint study record: Skin irritation / corrosion.001 by JP-AIST

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Study period From 2010-11-24 To 2011-1-25
Reliability 2 (reliable with restrictions)
Rationale for reliability The test was conducted by GLP-certified laboratory as a non-GLP study.

Data source

Reference

Reference type study report
Author Kohei Mizuno
Year 2011
Title Acute dermal irritation/corrosivity test of titanium dioxide nanoparticle E in rabbits

Testing laboratory Nippon Experimental Medical Research Institute Co., Ltd.
Report no. H-10195
Owner company AIST Japan
Company study no. H-10195
Report date 2011-01-24

Data access
data submitter is data owner

Data protection claimed
yes, but willing to share

Materials and methods

Type of method
in vivo

Test guideline
Qualifier according to
Guideline OECD Guideline 404 (Acute Dermal Irritation / Corrosion)
Deviations no

Principles of method if other than guideline
The protocol stipulated that the primary skin irritation index (P.I.I.) was to be calculated by first combining the scores obtained for erythema and edema at 24 and 72 hours after patch removal, and then dividing the total by [the number of animals × the number of tests performed (3 × 2 = 6)]. However, in the actual study, the primary skin irritation index (P.I.I.) was calculated by first combining the scores obtained for erythema and edema at 24, 48, and 72 hours after patch removal, and then dividing the total by [the number of animals × the number of tests performed (3 × 3 = 9)].

GLP compliance
no

Test material equivalent to submission substance identity
yes

Test materials
Test material identity
Identifier IUPAC name
Identity Titanium dioxide

Details on test material
Aeroxide P25 (principal sample)

Test animals
Species
rabbit

Strain
other: male Japanese White rabbits (Kbs:JW, Healthy)
Details on test animals and environmental conditions
TEST ANIMALS - Source: Kitayama Labes co ltd, Japan - Age at study initiation: 10-11 weeks - Weight at study initiation: 1.99-2.34 kg - Housing: Individually housed in an aluminum rabbit cage - Diet (e.g. ad libitum): Solid pellets, ad libitum - Water (e.g. ad libitum): Drink tap water, ad libitum - Acclimation period: 15 days ENVIRONMENTAL CONDITIONS - Temperature (°C): 19.8-20.7°C - Humidity (%): 41-64% - Air changes (per hr): More than 10 times per hr - Photoperiod (hrs dark / hrs light): 12 hours light IN-LIFE DATES: From: To:

Test system

Type of coverage
occlusive

Preparation of test site
shaved

Vehicle
water

Amount/concentration applied
TEST MATERIAL 0.5 g of the test material was evenly distributed onto a 2.5 x 2.5 cm lint patch and was applied to the skin. VEHICLE The patches were slightly moisturized by water mist to ensure good skin contact.

Duration of treatment / exposure

0, 3, 60, and 240 hours for the initial test and 240 hours for the confirmatory test.

Observation period

1, 24, 48, and 72 hours after the exposure.

Number of animals

One for the initial test and two for the confirmatory test.

Control animals

yes, concurrent no treatment

Details on study design
TEST SITE - Area of exposure: 2.5 x 2.5 cm - % coverage: - Type of wrap if used: The lint patch was covered with a 3.5 x 3.5 cm elastic adhesive bandage, and was held by wrapping the trunk of the animal using Silkytex and surgical tapes in an occlusive manner. REMOVAL OF TEST SUBSTANCE - Washing (if done): Gently wiped with an absorbent cotton ball soaked with water for injection (Fuso Pharmaceutical Industries, Ltd). - Time after start of exposure: SCORING SYSTEM: (1) Skin reaction criteria stipulated in the OECD guidelines. (2) Primary skin irritation index (P.I.I.)

Any other information on materials and methods incl. tables

In order to prevent photocatalytic activity of thd test material, administration was carried out in an area protected from light in the housing room.

Results and discussions

Irritation / corrosion results

Irritation parameter	erythema score
Basis	mean
Time point	1,24,48, and 72 hours
Score	0
Max. score	0
Reversibility	
Remarks	
Irritation parameter	primary dermal irritation index (PDII)
Basis	mean
Time point	1,24,48, and 72 hours
Score	0
Max. score	0
Reversibility	
Remarks	

Irritant/corrosive response data

No irritatant/corrosive reaction was observed at any observation time point of any animal.

Overall remarks, attachments

Attached background material

H-10195_Results.xls

Irritant response data for each animal at each time point

Animal		Observation time point (hours)			
		1	24	48	72
1	Erythema	0	0	0	0
	Edema	0	0	0	0
	Other	—	—	—	—
	Total	0	0	0	0

2	Erythema	0	0	0	0
	Edema	0	0	0	0
	Other	—	—	—	—
	Total	0	0	0	0
3	Erythema	0	0	0	0
	Edema	0	0	0	0
	Other	—	—	—	—
	Total	0	0	0	0
Mean score	Erythema	0,0	0,0	0,0	0,0
	Edema	0,0	0,0	0,0	0,0
	Total	0,0	0,0	0,0	0,0

P.I.I. : 0.0 (Non irritant)

Applicant's summary and conclusion

Interpretation of results

other: Non irritant

Criteria used for interpretation of results

other: Draize skin irritation criteria

Conclusions

No skin irritation was observed at any of the observation time points in any animal after patch removal. Moreover, no corrosivity was observed at any observation time points in any animal.

7.3.2 Eye irritation

Endpoint study record: Eye irritation.001 by JP-AIST

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Study period From 2011-11-24 To 2011-1-25
Reliability 2 (reliable with restrictions)
Rationale for reliability The test was conducted by GLP-certified laboratory as a non-GLP study.

Data source

Reference

Reference type study report
Author Kohei Mizuno
Year 2011
Title Acute ocular irritation/corrosivity test of titanium dioxide nanoparticle E in rabbits
Testing laboratory Nippon Experimental Medical Research Institute Co., Ltd.
Report no. H-10196
Owner company AIST Japan
Company study no. H-10196
Report date 2011-01-24

Data access
data submitter is data owner

Data protection claimed
yes, but willing to share

Materials and methods

Type of method

in vivo

Test guideline

Qualifier according to
Guideline OECD Guideline 405 (Acute Eye Irritation / Corrosion)
Deviations no

GLP compliance

no

Test material equivalent to submission substance identity

yes

Test materials

Test material identity

Identifier IUPAC name
Identity titanium(IV) dioxide

Details on test material

Aeroxide P25 (Principal material)

Test animals

Species
rabbit

Strain
other: Japanese White rabbit (Kbs:JW, Healthy)

Details on test animals and environmental conditions
TEST ANIMALS - Source: Kitayama Labes co ltd - Age at study initiation: 13-14 weeks - Weight at study initiation: 2.6-3.3 kg - Housing: individually housed in aluminum rabbit cage - Diet (e.g. ad libitum): solid pellets, ad libitum - Water (e.g. ad libitum): drink tap water, ad libitum - Acclimation period: 1 day ENVIRONMENTAL CONDITIONS - Temperature (°C): 19.8-20.3 - Humidity (%): 47.8-63.1 - Air changes (per hr): more than ten times - Photoperiod (hrs dark / hrs light): 12 hours light IN-LIFE DATES: From: To:

Test system

Vehicle
unchanged (no vehicle)
Amount/concentration applied
0.1 g of the test material was instilled in the conjunctival sac.

Duration of treatment / exposure
After the instillation of the test material into the conjunctival sac, the upper and lower lids were gently held together for about 1 second.

Observation period
1, 24, 48, 72 hours after the instillation

Number of animals
One for the initial test and two for the confirmatory test

Control animals
yes, concurrent no treatment

Details on study design
REMOVAL OF TEST SUBSTANCE - Washing (if done): none - Time after start of exposure: SCORING SYSTEM: Mean Total Score (MTS) in accordance with the Kay & Calandra classification for eye irritation. TOOL USED TO ASSESS SCORE: hand-slit lamp

Any other information on materials and methods incl. tables
Sample preparation was carried out under yellow light to reduce photocatalytic activity.

Results and discussions

Overall irritation / corrosion results

Irritation parameter	overall irritation score
Basis	mean
Time point	1 hour

ca. 7.3

Max. score

Reversibility

Remarks

Irritation parameter overall irritation score

Basis mean

Time point 24 hour

ca. 1.3

Max. score

Reversibility

Remarks

Irritation parameter overall irritation score

Basis mean

Time point 24 and 72 hour

ca. 0

Max. score

Reversibility

Remarks

Irritant/corrosive response data
See attached material

Overall remarks, attachments

Attached background material

Attached document 18: H-10196 response data.pdf: ENV/JM/MONO(2015)17/PART1/ANN21

Applicant's summary and conclusion

Interpretation of results

other: Minimally irritating : M1

Criteria used for interpretation of results

other: Kay & Calandra, J. Soc. Cosm. Chem.,281-289, 1962.

7.4 Sensitisation

7.5 Repeated dose toxicity

7.5.1 Repeated dose toxicity: oral

Summary of the HH literature data status 03rd April 2014 : Summary of studies on repeated dose toxicity (oral route)

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
RDT – oral route						
Trouiller et al. 2009	P25	mouse	Oral (drinking water)	500 mg/kg, 5 d	POD = 500 mg/kg	P25 induced a proinflammatory response in peripheral blood as revealed by altered cytokine mRNA levels.

7.5.2 Repeated dose toxicity: inhalation

Summary of the HH literature data status 03rd April 2014 : Summary of studies on repeated dose toxicity (inhalation route)

Short term exposure

Reference	Material/ Size	Test Organism (Strain)/ Test System	Exposure/ dose	Main findings
Short-term exposure				
Ferin et al. 1991	a. ultrafine P25 (20 nm) b. fine 250 nm (Fisher)	Rat (Fisher 344)	a. 25.5 mg/m^3 b. 21.8 mg/m^3 (6 h/d for 10 d)	At comparable mass lung burden, only P25 induced an inflammatory response with increased PMN numbers, which, however, was milder than in case of bolus administration. In contrast to intratracheal instillation there was no enhanced translocation in case of smaller particles even at three times higher final total lung burden in case of inhalation. This was attributed to the effective removal by AM as long as the lungs are not overwhelmed.
van Ravenzwaay et al. 2009	a. P25 b. pigmentary TiO_2 (rutile, 200 nm, Kronos)	Rat (Wistar (strain Crl:WI (Han))	a. 100 mg/m^3 b. 250 mg/m^3 (6 h/d for 5d)	Mild and reversible neutrophilic inflammation. Partially reversible activation of lung macrophages according to BAL parameters and histological examination after 14 d post-exposure. Nanoparticle effects were slightly more pronounced.
Ma-Hock et al. 2009	P25	Rat (Wistar (strain Crl:WI (Han))	0, 2, 10 and 50 mg/m^3 (6 h/d for 5d)	Dose-dependent inflammatory effects in a number of BAL parameters and cell counts. Lung cell proliferation by BrdU labeling already at 2 mg/cm^3 with incomplete recovery 16 d p.e. Histopathological changes in the lung at 10 mg/cm^3 and

				above but no systemic effects.

subacute

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
RDT – inhalative (subacute)						
Creutzenberg et al. (2009) [abs..]	a. P25 (anatase/ rutile, pps: 21 nm, MMAD: 0.7 µm) Bayertitan (rutile, pps: 0.3 µm, MMAD: 1.1 µm)	rat	Inhalation	21 d (6h/d) nose-only a. 2 and 10 mg/m3 b. 9 and 45 mg/m3) Recovery time points: 3, 28, 90 d p.e.	NOAEC = 2 mg/m3 mg/m3 (NP)	Haematology revealed decreases in white blood cells in the high-dose (overload) groups at days 28 and 90 for both dusts and on day 3 for P25 only. This effect was thus persistent and was more pronounced for P-25. TiO2-inhalation even at overload did not alter levels of PMN in BALF.
Eydner et al. 2012	b. P25 (anatase/ rutile, pps: 21 nm, MMAD: 0.7 µm) c. Bayertitan (rutile, pps: 0.3 µm, MMAD: 1.1 µm)	rat	Inhalation (OECD 412, GLP)	21 d (6h/d) nose-only a. 10 mg/m3 b. 45 mg/m3) Recovery time points: 3, 28, 90 d p.e.	LOAEC > 10 mg/m3 (NP)	A relevant change in BAL parameters was not found for either particle type, despite an inconsistent decreased in activity of beta-glucoronidase (used as a marker for phagocytic activity)..
Creutzenberg et al., 2012 a	P25 (MMAD approx. 0.8 µm, GSD: 1.8 µm)	rat	Inhalation (nose-only)	21 d (10 mg/m3)		Under exposure conditions used (not detailed), approx. 1.4 mg P25/lung was retained. Histopathology and BAL resulted in insighnificant inflammation after 3d p.e.

Sub-chronic exposure

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
RDT – inhalative (subchronic)						
Ferin et al. 1992	a. P25 b. 250 nm (Fisher)	rat	Inhalation	~23 mg/m^3, (6 h/d, 5d/wk for 90 d)	POD = 23 mg/m^3	The more efficient interstitial translocation of P25 was accompanied by an acute transient inflammatory response as revealed by increased PMN cell numbers in BALF.
Oberdörster et al. 1994a	a. P25 b. 250 nm (Fisher)	rat	Inhalation	a. 23.5 mg/m^3 b. 22.3 mg/m^3 (6 h/d, 5 d/wk for 12 wks)	POD = 23 mg/m^3	P25 induced a progressive increase in total cell and PMN numbers (and lavageable protein), which persisted almost a year p.e. Retained (for 7 months) impairment test particle clearance function of alveolar macrophages in case of P25. Histology revealed early fibrotic reactions (reversible) and type II cell hyperplasia in case of P25. Altogether, nanoparticles had a higher pulmonary inflammatory potency than pigment particles which was attributed rather to their relatively larger surface area than to particle volume.
Oberdörster et al. 1994b	a. P25 b. 250 nm (Fisher)	rat	Inhalation	a. 23.5 mg/m^3 b. 22.3 mg/m^3 (6 h/d, 5 d/wk for 12 wks)	POD = 23 mg/m^3	At comparable volumetric loading, P25 particles caused greater pulmonary inflammation, penetrated more readily into the interstitium (accumulation in lymph nodes), and led to greater impairment of particle clearance and a higher degree of (mild) fibrosis than pigment particles. An additional surface area-based effector mechanism was hypothesised to the volumetric impairment of AM clearance function.
Baggs	a. P25	rat	Inhalation	a. 23.5	POD =	Development of interstitial

et al. 1997	b. 250 nm (Fisher)			mg/m^3 b. 22.3 mg/m^3 (6 h/d, 5 d/wk for 12 wks)	23 mg/m^3	fibrosis within 6 months (more pronounced in case of P25) that largely returned to control levels after 1 yr p.e.
Bermudez et al. 2004	P25	rat mouse hamster	Inhalation	0.5, 2.0, 10 mg/m^3 6h/d, 5h /wk, 13 wks	LOAEC = 2 mg/m^3	Markedly impaired particle clearance from lung and pulmonary inflammatory response in mice and - more severe - in rats but not in hamsters. Only rats showed progressive epithelial changes including metaplasia in the high dose group. Alveolar cell proliferation and histopathological lesions already evident at mid dose level.

Chronic exposure

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
RDT – inhalative (chronic)						
Creutzenberg et al. 1990;	P25	rat	Inhalation	7.5 mg/m^3 for 4 months, followed by 15 mg/m^3 for 4 months and 10 mg/m^3 for 14 months (19h/d, 5d/wk)	LOAEC = ~10 mg/m^3	Substantial increase in lung weight over time (peaking at 18 months of exposure) and histopathology indicated pronounced proliferative response of lung tissue. Lung burden after 2 yrs was 39.3 mg indicating massive overload. Tracer (^{85}Sr polystyrene) half-time of ~ 500d indicated collapse of clearance functions.
Muhle et al. 1990	P25	rat	OECD 453 Inhalation	7.2 mg/m^3 for 4 months, 14.8 mg/m^3 for 8 mo, 9.4 mg/m^3 for 16 mo (95h/wk)	LOAEC = ~10 mg/m^3	Interstitial fibrosis at 12 and 18 months. Lung weight increased by 4.25 after 22 months of exposure accompanied by disturbed lung function, shallower breathing and altered particle deposition pattern.

7.5.3 Repeated dose toxicity: dermal

Summary of the HH literature data status 03rd April 2014 : Summary of studies on repeated dose toxicity (drmal route)

Reference	Material/ Size	Test Organism / System	Method	Exposure/ dose	Derived effect value (dose descriptor)	Main findings
RDT – dermal route						
Wu et al. 2009	P25	mouse (hairless)	Topical (semi-occluded)	400 µg/cm^2 per day, 60 d	POD = 400 µg/cm^2	Induction of various tissue damages, most notably in skin and liver accompanied by signs of oxidative stress (elevated superoxide dismutase and malondialdehyde as well as reduced hydroxyproline levels).

Endpoint study record: van Ravenzwaay (publication)

Administrative Data

Purpose flag weight of evidence ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Reliability 3 (not reliable)
Rationale for reliability Method not validated

Data source

Reference

Reference type publication
Author van Ravenzwaay B, Landsiedel R, Fabian E, Burkhardt S, Strauss V, Ma-Hock L
Year 2009
Title Comparing fate and effects of three particles of different surface properties: Nano-TiO2, pigmentary TiO2 and quartz
Bibliographic source Toxicology Letters 186 (2009) 152–159

Materials and methods

Test type

other: short-term

Limit test

no

Test guideline

Qualifier no guideline available

Guideline

Deviations

Principles of method if other than guideline
Short-term (5d) inhalation study using high exposure dose (100 mg/cm3)

GLP compliance
no data

Test materials

Test material equivalent to submission substance identity
no

Test material identity

Identifier CAS number
Identity 13463-67-7

Details on test material
- anatase/rutile (70/30) – uncoated - PPS: 20-30 nm - PSD: approx. 10% of particles < 100 nm corresponding to 0.5% of total particle mass - BET surface area: 48.6 m2/g - Zeta Potential: IEP pH7 (10c mM KCl)

Test animals

Species
rat

Strain
Wistar

Sex
male

Administration / exposure

Route of administration
inhalation: aerosol

Type of inhalation exposure
nose/head only

Vehicle
water

Analytical verification of doses or concentrations
yes

Duration of treatment / exposure

5d Post exposure period: up to 14 d

Frequency of treatment
6h/d

Doses/concentrations
100 mg/m3
Basis nominal conc.
88.0+/- 6.4 mg/m3
Basis analytical conc.

MMAD / GSD
1.0/2.2

Examinations

Any other information on materials and methods incl. tables
1. Tissue distribution (immediately after last exposure and 14 d after exposure) 2. BAL: cells, protein, enzymes (immediately after last exposure as well as 3 and 14 d after exposure) 3. Histochemistry: LM/TEM (immediately after last exposure and14 d after exposure)

Results and discussions

Effect levels
Endpoint conc. level:
Effect level 100 mg/m³ air (nominal)
Sex male
Basis for effect level / Remarks Mild inflammation (histopathology, BAL)

Observations

Remarks on results including tables and figures
Particles detected in lungs and LALN only. No particles detected in liver, kidney, spleen and basal brain with olfactory bulb.Translocation to mediastinal lymph nodes but at smaller amounts as for 200 nm particles (tested in the same study). No substantial difference in deposition, distribution and toxicity between nanoparticles and larger particles. This was attributed to the strong agglomeration behaviour of the nanoaparticles. Aleveolar macrophages with internalised clustered nanoparticles were demonstrated by TEM. Mild and reversible neutrophilic inflammation and activation of lung macrophages for either particle type according to BAL parameters and histological examination.

Overall remarks, attachments

Attached background material
()

Attached full study report
()

Applicant's summary and conclusion

Executive summary
Non-guideline inhalation study using one high aerosol concentration. Material identity not described, though related studies suggested that P25 was used (refer to cross-references). Test material

characterisation appropriate. Information on tissue distribution implied translocation confined to lung compartment and associated lymph nodes. However, detection limit for Ti was 0.5 µg/organ sample. Comparability to studies of longer exposure duration need further examination: High particle concentration in aerosol results in heavy agglomeration. Pulmonary clearance mechanism of large agglomerates may considerably differ from that of small aggregates and singular nanoparticles which in turn may affect translocation. This may also explain the rapid reversibility of the inflammatory response not observed in rats using lower-concentrated subchronic aerosol exposure.

Cross-reference to other study

1. Ma-Hock L, Burkhardt S, Strauss V, Gamer A, Wiench K, van Ravenzwaay B, Landsiedel, R. (2009). Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance. Inhalation Toxicology 21(2), 102-118. 2. NanoCare (2009). Health related Aspects of Nanomaterials. Final Scientific Report. DECHEMA

Endpoint study record: by Fraunhofer ITEM

Administrative Data

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

Data source

Reference

Reference type study report
Author Otto Creutzenberg
Year 2013
Title Toxic Effects of Various Modifications of a Nanoparticle Following Inhalation
Bibliographic source BAuA Research Project F 2246
Testing laboratory Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) Nikolai Fuchs Strasse 1 30625 Hannover, Germany
Report no. Fraunhofer ITEM Study No. 02 N 11 538
Owner company Federal Institute for Occupational Safety and Health

Materials and methods

Test guideline
Qualifier according to
Guideline OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)
Deviations yes 90d postexposure study

Test animals

Species
rat

Strain
Wistar

Sex
male

Details on test animals and environmental conditions

Male Wistar rats [strain Crl:WI (Han)] were purchased from Charles River Deutschland (Sulzfeld, Germany). The age of the animals at the start of exposure was approx. 8 weeks and the weight approx. 270 gram. Rats were exposed to the test item by nose-only inhalation. For a period of 2 - 3 weeks prior to exposure animals were trained to become accustomed to nose-only tubes.

Administration / exposure

Route of administration

inhalation: aerosol

Type of inhalation exposure

nose only

Duration of treatment / exposure

Rats were exposed to aerosol concentrations (low, mid, high) of 3, 12 and 48 mg/m3 for 28 days (6 hours/day, 5 days/week) while concurrent controls inhaled clean air. This dosing scheme was aiming at achieving non-overload, partial overload and complete overload conditions in the low, mid and high dose groups, respectively. Subsequently, endpoints were analysed at day 3, 45 and 94 of the post-exposure period. period. Calculation: According to the MPPD model the deposition rate for particles with an agglomerate density ρAgg = approx. 1.7 and an MMAD of approx. 1 µm amounts to approx. 7%. In a 28-day test the retained particles masses would result in approx. 0.20, 1.0 and 6 mg/lung, respectively.

Details on study design

The particulate sample aerosols were generated by dry dispersion with pressurized air. Dispersion was achieved by a feeding system and a high-pressure, high-velocitypressurized air dispersion nozzle developed by Fraunhofer ITEM (Koch, 1998). For each nose-only exposure unit, the aerosol was generated by a high-pressure pneumatic disperser. The disperser was fed with the test/reference items under computerized control, i.e. with a feed back loop to the actual aerosol concentrations measured by an aerosol photometer (see Figure 3.3). The photometer gives a scattering light signal which is proportional to the particle concentration, if the particle size distribution is constant. The ratio between photometer signal and concentration was determined throughout the study by comparing to gravimetric concentrations. The aerosol was given to the rats by a flow-past nose-only inhalation exposure system which was used for previous particle and fiber inhalation studies at Fraunhofer ITEM. In this system, aerosols were supplied to each rat individually, and exhaled air was immediately exhausted. The airflow to each rat was approximately 1 l/min which is calculated to be laminar. Therefore measurement of the oxygen concentration is not necessary. Prior to the 28-day exposure of rats, technical trials to adjust particle size distributions and exposure levels were conducted. Additionally, the mass median aerodynamic diameter (MMAD) was determined 2-3 times using a cascade impactor (Marple impactor). Filter samples of the aerosols were taken daily to control the aerosol concentrations and to calibrate the aerosol photometers. These samples were collected at a port of the nose-only exposure unit, thus, under the same conditions the rats are inhaling the aerosol. In Table 3.3, the means of the aerosol concentrations are summarized comprising the exposure period from September 19 to October 18, 2011. Each animal was exposed for 20 days. The means are close to the target concentrations.

Examinations

Sacrifice and pathology

All animals were subjected to a complete necropsy, which included a careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. For anesthesia an overdose of carbon dioxide was used. The abdominal cavity was opened and the diaphragm

was cut carefully allowing the lungs to collapse. Heart, esophagus, upper half of trachea, thymus and lung associated lymph nodes (LALN) were removed from the lung convolution. The lung was inflated under a pressure of about 20 cm water with formalin and was fixed by immersion for a minimum of 2 hours, and used for histopathology. Thereafter the weight of the lower part of the trachea was recorded and the weight of the lung was calculated. The following organs were trimmed and wet weights were recorded: liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain, and heart. All tissues listed in OECD Guideline no. 412, table 2 were prepared for histopathology. The trimming was done according to Ruehl-Fehlert et al. (2003), Kittel et al. (2004) and Morawietz et al. (2004).

Other examinations

The following histopathology was performed in 6 animals per group after end of exposure (day 3) and in the recovery group animals on day 45 and day 94 after exposure: · full histopathology on the respiratory tract and other organs and tissues, as listed in OECD 412 of all animals in the clean air control group and the high dose groups and all animals that died or were killed during the study. · histopathology of the left lung lobe, including bronchi and the lung-associated lymph nodes (LALN), trachea, larynx, pharynx, the nasal cavities (turbinales) and visceral pleura in all animals of all groups. Lungs were fixed in buffered formalin (10%), embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E). A special stain was applied for diagnosis of fibrotic changes: Masson trichrome. After sacrifice the right lung lobes were used for transmission electron microscopy (TEM) analysis.

Statistics

Differences between groups were considered statistically significant at $p < 0.05$. Data were analyzed using analysis of variance. If the group means differed significantly by the analysis of variance the means of the treated groups were compared with the means of the control groups using Dunnett's test. The statistical evaluation of the histopathological findings will be done with the twotailed Fisher test by the PROVANTIS system. If necessary, further statistical procedures will be applied upon agreement with the sponsor.

Results and discussions

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption

not examined

Food efficiency

not examined

Water consumption

not examined

Ophthalmoscopic examination

not examined

Haematology

yes

Clinical chemistry
yes

Urinalysis
not examined

Neurobehaviour
not examined

Organ weights
yes

Gross pathology
yes

Histopathology: non-neoplastic
yes

Histopathology: neoplastic
not examined

Details on results
see attachment

Overall remarks, attachments

Attached full study report
Attached document: 02N11358_300912_final_090413.pdf (general annex): ENV/JM/MONO(2015)17/ANN21

7.6 Genetic toxicity

7.6.1 Genetic toxicity in vitro

Summary of the HH literature data status 03rd April 2014 : Summary of studies on Genotoxicity in vitro

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Genotoxicity – in vitro					
Rahman et al. 2002	a. Ultrafine TiO_2 (20 nm, P25?) b. fine TiO_2 (200 nm)	Syrian hamster embryo (SHE) cells (hamster)	Micronucleus test	1.0 µg/cm^2 for 12 to 72 h	Ultrafine TiO_2 induced weak but significant induction of micronuclei, possibly by disturbed chromosome segregation in mitosis, and apoptosis
Kang et al. 2008b	P25	Peripheral blood lymphocytes (human)	1. Alkaline comet assay 2. CBMN	20, 50, or 100 mg/ml (1. 0-24h 2. 72h)	Significantly increased micronucleus formation and DNA breakage in a dose-dependent manner. Evidence that ROS

					activation of specific p53 signalling pathway is involved in apoptosis.
Shi et al. 2009	P25	L-02 hepatocytes (human)	1. Comet assay (alkaline and neutral) 2. Micronucleus test 3. 8-OHdG	0 – 1 µg/ml (24h)	P25 was negative in the comet and micronucleus assay but produced 8-OHdG adducts. However, it synergistically increased DDP-induced formation of 8-OHdG adducts, DNA breaks and chromosomal damage.
Barillet et al. 2010	a. P25 b. "TiO_2-CEA" (95% anatase, 12 nm PPS)	NRK-52E kidney proximal cell line (rat)	γ-H2AX foci (immunostaining) Comet assay	20-200 µg/ml (24 h)	P25 did not induce double strand breaks in the H2AX assay. TiO_2-CEA proved positive in the Comet assay showing dose-dependence but no clear correlation to ROS production.
Jugan et al. 2012	P25 and other nanoparticles (size range 12 – 140 nm, anatase or rutile).	A549 lung carcinoma cells (human)	1. Comet assay 2. Micronucleus assays 3. γ -H2AX immunostaining, 4. 8- OHdG analysis, 5. H2-DCFDA, 6. glutathione content, antioxidant enzymes activities	50-200 µg/ml up to 48 h (depending on test)	positive (small and spherical NP) ROS generation Oxidative DNA damage, including single-strand breaks and 8-OHdG, but not double-strand breaks or chromosomal breaks or losses. NPs impaired cell ability to repair damage to DNA.

Endpoint study record: Genetic toxicity in vitro.001 by JP-AIST

Administrative Data

Purpose flag ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Study period From 2011-3-2 To 2011-4-20
Reliability 1 (reliable without restriction)
Rationale for reliability The Standards for Testing Laboratories that Implement Tests of New Chemical Substances [PFSB Notification No. 1121003, dated November 21, 2003; METI Notification No. 3, dated November 17, 2003; and EPB Notification No. 031121004, last updated on July 4, 2008] issued by the MHLW, the METI, and the MOE.

Data source

Reference

Reference type study report
Author Kohei Mizuno
Year 2011
Title Reverse mutation test of titanium dioxide nanoparticle E in bacteria
Testing laboratory Nippon Experimental Medical Research Institute Co., Ltd.
Report no. H-11018
Owner company AIST Japan
Company study no. H-11018

Report date 2011-04-19

Data access

data submitter is data owner

Data protection claimed
yes, but willing to share

Materials and methods

Type of genotoxicity

gene mutation

Type of study
bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier according to
Guideline JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals
Deviations no

GLP compliance
yes

Test materials

Test material equivalent to submission substance identity
yes

Test material identity

Identifier IUPAC name
Identity titanium(IV) dioxide

Details on test material
Aeroxide P25 (principal material)

Method

Species/strain

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	with and without
Metabolic activation system	S9 mix
Species/strain	E. coli WP2
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	with and without
Metabolic activation system	S9 mix

Test concentrations

5000, 2500, 1250, 625, and 312.5 µg/plate

Controls

Negative controls
Solvent / vehicle controls yes
True negative controls
Positive controls yes
Positive control substance other: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, Sodium azide, 9-Aminoacridine, and 2-Aminoanthracene
Remarks

Any other information on materials and methods incl. tables

Test material was dispersed in DMSO in concentration of 50 mg/mL with 10-minites sonication and used for the testing after stepwise dilution.

Results and discussions

Test results

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	
Vehicle controls valid	yes
Negative controls valid	
Positive controls valid	yes
Species/strain	E. coli WP2
Metabolic activation	with and without

Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	
Vehicle controls valid	yes
Negative controls valid	
Positive controls valid	yes

Remarks on results including tables and figures

Precipitation of test substance was observed at 1250 µg/plate and higher doses.

Overall remarks, attachments

Attached background material

H-11018 Results.xls

Test material: Evonik P25

Test date			From 28-Mar-2011 To 1-Apr-2011				
Presence/absence of metabolic activation	Dose (μg/plate)		Number of revertant colonies (numbers per plate)				
			Base substitution			Frame shift	
			TA100	TA1535	WP2*uvrA*	TA98	TA1537
−	S9 mix		105	11	17	21	5
		Negative control (DMSO)	89 (96)	11 (10)	19 (17)	15 (17)	7 (6)
			95	9	16	16	5
			104	10	18	15	6
		312,5	97 (101)	10 (10)	19 (19)	15 (15)	10 (8)
			122	6	15	19	4
		625 †	97 (110)	4 (5)	19 (17)	18 (19)	3 (4)
			102	10	16	22	5
		1250 †	99 (101)	10 (10)	17 (17)	16 (19)	3 (4)
			91	5	20	16	4
		2500 †	113 (102)	7 (6)	15 (18)	16 (16)	6 (5)
			104	5	12	17	5
		5000 †	83 (94)	7 (6)	19 (16)	21 (19)	8 (7)
＋	S9 mix		110	11	24	40	7
		Negative control (DMSO)	110 (117)	12 (13)	25 (24)	34 (37)	11 (10)
			132	15	23	37	11
			129	10	24	28	9
		312,5	118 (124)	12 (11)	30 (27)	30 (29)	7 (8)
			130	5	20	31	6
		625 †	135 (133)	8 (7)	31 (26)	38 (35)	8 (7)
			113	10	26	26	10
		1250 †	135 (124)	11 (11)	24 (25)	37 (32)	14 (12)
			100	10	18	38	13
		2500 †	113 (107)	7 (9)	21 (20)	30 (34)	9 (11)
			115	7	15	33	5
		5000 †	108 (112)	7 (7)	15 (15)	27 (30)	6 (6)
Positive control	Those which does not need S9 mix	Substances	AF−2	SA	AF−2	AF−2	9−AA
		Dose (μg/plate)	0,01	0,5	0,01	0,1	80
		Number of colonies per plate	404	413	177	475	273
			363 (384)	393 (403)	152 (165)	521 (498)	246 (260)
	Those which need S9 mix	Substances	2−AA	2−AA	2−AA	2−AA	2−AA
		Dose (μg/plate)	1	2	10	0,5	2
		Number of colonies per plate	1012	261	914	558	315
			1037 (1025)	246 (254)	991 (953)	540 (549)	336 (326)

Legends:
( ): Mean value
†: Precipitation of test material
DMSO : Dimethyl sulfoxide
AF-2 : 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide、SA : Sodium azide、9-AA : 9-Aminoacridine、
2-AA : 2-Aminoanthracene

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Genetic toxicity in vitro by University of Alberta

Administrative Data

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

Data source

Data access

other: performed and provided by University of Alberta, Canada

Cross-reference to same study
"In vitro cytotoxicity and genotoxicity studies of titanium dioxide (TiO2) nanoparticles in Chinese hamster lung fibroblast cells" Mahsa Hamzeh, Geoffrey I. Sunahara Toxicology in Vitro 27 (2013) 864–873

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study
other: Comet Assay

Test materials

Details on test material
nominal size measured: 34.1nm agglomeration/ aggregation: 400nm (DLS) Zeta-Potential: -20.1 mV in water, -12 mV (pH 8), -16.2 mV (pH 6.5) Sample is dispersed by homogenization (stock solutions in the range of 400-800 ppm in culture medium) at 5000 rpm for 5 min using a IKA® T25 digital Ultra-Turrax homogenizer (IKA® Works Inc., Wilmington, NC, USA). Samples were used the same day of homogenization and vortexed before use to have as homogeneous solution as possible, since the sample will sediment out with time. BET Surface Area measured: 48.9 m2/g

Method

Test concentrations
1, 10, 100 mg/L

Vehicle
For mammalian cells (V79) the medium used was DMEM.

Details on test system and conditions
Chinese hamster lung fibroblast (V79) Exposure duration: 24 and 48 hours The comet assay kit was supplied by Trevigen (Gaithersburg, MD) and the experiment was carried out according to the manufacturer's protocol. Number of replicate: 3 replicates and 3 separate experiments. Frequency of Dosing: Just once Positive and negative control groups and treatment: Positive groups are cells exposed to hydrogen peroxide (H2O2), and negative groups are cells in the absence of nanoparticles.

Cells were evaluated per dose group and per different types of TiO2 exposed group.

Statistics
One way ANOVA followed by a post-hoc test.

Results and discussions

Additional information on results
P25 showed genotoxicity effect significantly compared to the control at 100 mg/L after 24h exposure.

Remarks on results including tables and figures
see attached publication

Overall remarks, attachments

Overall remarks

The present study shows the genetic effects of different nano-TiO2 particles on V79 cells using the comet assay. Our genotoxicity findings agree with earlier studies in the induction of DNA damage in cells after exposure to nano-TiO2, and a stronger induction was observed in nano-sized anatase compared to rutile.

Attached full study report

Hamzeh_Sunahara_ in vitro cytotox genotox.pdf: http://www.sciencedirect.com/science/article/pii/S0887233312003566?via=ihub: ENV/JM/MONO(2015)17/PART1/ANN24

Endpoint study record: Genetic toxicity in vitro_NM 105_COMET 3D skin by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	BfR (GER°	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: P25

Method

Species/strain

Species/strain other: human reconstructed full thickness skin model (MaTek) EpiDermFT (hRS)

Details on mammalian cell lines (if applicable)

Additional strain characteristics

Metabolic activation No metabolic activation

Metabolic activation system

Test concentrations

82/164/246 µg/cm²

Details on test system and conditions

single dose with incubation time of 72h

Evaluation criteria

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

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailed protocol in annex

Applicant's summary and conclusion

Interpretation of results

No cytotoxic effects

Negative: No increase in the % Tail DNA irrespective of the tested dose

Conclusions

TiO2 NM 105 does not induce DNA strand breaks in human reconstructed skin model following 72h treatment with the alkaline comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM_105_COMET 16-HBE by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	BfR (GER)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available
Guideline
Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial
Identity NM 105

Details on test material

Commercial name: P25

Method

Species/strain

Species/strain	mammalian cell line, other:
Details on mammalian cell lines (if applicable)	human bronchial epithelial 16 HBE cells
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

2/8/32/128/512 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h

Evaluation criteria

percentage of DNA in the tail (% Tail DNA) with 300 cells scored per dose

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Negative at 3 h and 24 h: no increase in the % Tail DNA at the tested dose
Positive at 24h: Statistical significant increase in the % Tail DNA at the highest dose. Dose-dependant increase

Conclusions

TiO2 NM 105 induces DNA strand breaks in 16-HBE cells following 24h but not 3 h with the alkaline comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_COMET A549 by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	NIOM (PL)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: P25

Method

Species/strain

Species/strain	mammalian cell line, other:
Details on mammalian cell lines (if applicable)	human alveolar epithelial A549 cells
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

50/100/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h

Evaluation criteria

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

Statistics

ANOVA test with Dunnett's post-hoc test

Overall remarks, attachments

Attached full study report Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**
Remarks	Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Positive at 3h: dose-dependant increase in the % Tail DNA with significant statistical increase at the highest dose (256 µg/ml)
Negative at 24h: no increase in the % Tail DNA

Conclusions

TiO2 NM 105 induces DNA strand breaks in A 549 cells at 3 h but not 24 h at the tested dose with the alkaline comet assay

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_COMET_BEAS-2B by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	NIOM (PL)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain	mammalian cell line, other:
Details on mammalian cell lines (if applicable)	human bronchial epithelial BEAS 2B cells
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

50/100/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h

Evaluation criteria

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

Statistics

ANOVA test with Dunnett's post-hoc test

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Negative at 3h and 24 h: No increase in the % Tail DNA at the tested dose

Conclusions

TiO2 NM 105 does not induce DNA strand breaks in the BEAS-2B cells following 3h and 24h with the alkaline comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_COMET Caco-2 by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	NIOM (PL)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but not willing to share

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

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

Test concentrations

50/100/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h

Evaluation criteria

percentage of DNA in the tail (% Tail DNA) with 200 cells scored.

Statistics

ANOVA test with Dunnett's post-hoc test

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Negative at 3 h: no increase in the % Tail DNA at the tested dose
Positive at 24 h: increase in the % Tail DNA at 100 and 256 µg/ml with dose-dependance

Conclusions

TiO2 NM 105 induces strand breaks following 24 h but not 3 h incubation in Caco-2 cells with the alkaline comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_COMET NHEK by NANOGENOTOX

Administrative Data

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

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

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain mammalian cell line, other: Normal human epidermal keratinocytes (NHEK) from

	Lonza
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

15/33/65 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 3 h and 24 h

Evaluation criteria

percentage of DNA in the tail (% Tail DNA) with 200 cells scored.

Overall remarks, attachments

Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**
Remarks	Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Equivocal: increase in the % Tail DNA at both 3h and 24h exposure at one dose only.

Conclusions

TiO2 NM 105 induces an equivocal response in NHEK cells following both 3h and 24 h incubation with the alkaline comet assay

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_MLA TK by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

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

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

gene mutation

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier according to

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

Deviations

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain	mammalian cell line, other: L5178Y TK +/-mouse lymphoma cells
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	without
Metabolic activation system	

Test concentrations

32/64/128/256/312.5 , 625/1250/2500 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time 24 h

Evaluation criteria

Mutation frequency forSmall colonies + Large colonies (x106 cells)

Overall remarks, attachments

Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**
Remarks	Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: cytotoxic effects at 5000 µg/ml (RTG= 23 %)

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Negative: No increase in the frequency of mutations

Conclusions

TiO2 NM 105 is not mutagenic in L5178Y TK +/-mouse lymphoma cells at the tested doses
With the in vitro mammalian cell gene mutation test.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_MN 16-HBE by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	IPL (F)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

Guideline other guideline: micronucleus assay (OECD guideline 487)

Deviations yes Study without cytochalasin B

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain	mammalian cell line, other: human bronchial epithelial 16 HBE cells
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

64/128/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 41 h

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Negative: No increase in the frequency of binucleated cells with micronuclei

Conclusions

TiO2 NM does not induce aneugenic/clastogenic damage in 16-HBE cells at the tested dose with the Cytokinesis-block micronucleus assay

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_MN A549 by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	RIVM (NL)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

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

Deviations yes Cyto B added 6 h after NM

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain	mammalian cell line, other: human alveolar epithelial A549 cells
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

2/4/8/16/32/64/128/256/512 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square

Overall remarks, attachments

Attached background material

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailes porocol in annex

Attached document ()

Remarks

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:
- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):
- Without metabolic activation:

Negative: No increase in the frequency of binucleated cells with micronuclei

Conclusions

TiO2 NM 105 does not induce aneugenic/clastogenic damage in A 549 cells at the tested dose with the Cytokinesis-block micronucleus assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_MN Caco-2 by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	Anses (F)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

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

Deviations yes Cyto B added 24 h after NM

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

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

Test concentrations

9.5/28/85/128/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 52 h

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

No increase in the frequency of binucleated cells with micronuclei

Conclusions

TiO2 NM 105 does not induce aneugenic/clastogenic damage in Caco-2 cells at the tested dose with the Cytokinesis-block micronucleus assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_MN BEAS-2B by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	FIOH (FL)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier	according to
Guideline	other guideline: Cytokinesis-block micronucleus assay (OECD guideline 487)
Deviations	yes Cyto B added 6 h after NM

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier	Reference Material/Nanomaterial
Identity	NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain	mammalian cell line, other: human bronchial epithelial BEAS 2B cells
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

32/64/128/256 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 48 h

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Negative: No increase in the frequency of binucleated cells with micronuclei

Conclusions

TiO2 NM 105 does not induce aneugenic/clastogenic damage in BEAS-2B cells at the tested dose with the Cytokinesis-block micronucleus assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_MN Lymphocytes by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	INSA (PT)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

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

Deviations yes Cyto B added 6 h after NM

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain	primary culture, other: human primary peripheral blood lymphocytes
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

5/15/45/125/250 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 30 h with Cyto B added after 6 h exposure with NM

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Negative: No increase in the frequency of binucleated cells with micronuclei irrespective of the dose

Conclusions

TiO2 NM 105 does not induce aneugenic/clastogenic damage in human blood lymphocytes at the tested dose with the Cytokinesis-block micronucleus assay.

Overall remarks, attachments

Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**
Remarks	Data in the report and detailes porocol in annex

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vitro_NM 105_MN NHEK by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	H Norppa	**Year**	2013
Title	Deliverable 5: In vitro testing strategy for nanomaterials including database		
Bibliographic source			
Testing laboratory	IMB-BAS (BG)	**Report no.**	D5
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian cell micronucleus test

Test guideline

Qualifier according to

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

Deviations yes Cyto B added 6 h after NM

Test materials

Test material equivalent to submission substance identity

yes

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Details on test material

Commercial name: Aeroxid P25

Method

Species/strain

Species/strain	mammalian cell line, other: Normal human epidermal keratinocytes (NHEK) from Lonza
Details on mammalian cell lines (if applicable)	
Additional strain characteristics	
Metabolic activation	
Metabolic activation system	

Test concentrations

7.5/37.5/75 µg/ml

Vehicle

BSA 0.05 % prepared in milliQ water

Details on test system and conditions

single dose with incubation time of 54 h

Evaluation criteria

1000 cells scored per culture; 2000 cells scored per condition

Statistics

Chi square

Overall remarks, attachments

Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D5_in vitro: **ENV/JM/MONO(2015)17/ANN9**
Remarks	Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

Cytotoxic concentration:

- Without metabolic activation: No cytotoxic effects

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):

- Without metabolic activation:

Positive: Dose-dependant increase in the frequency of binucleated cells with micronuclei

Conclusions

TiO2 NM 105 induces aneugenic/clastogenic damage in NHEK cells with the Cytokinesis-block micronucleus assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

7.6.2 Genetic toxicity in vivo

Summary of the HH literature data status 03rd April 2014 : Summary of studies on Genotoxicity in vivo

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Genotoxicity – in vivo					
Gallagher et al. 1994	P 25	rat	Inhalation (^{32}P-post-labelling assay)	10.4 mg/m^3 (2 mo, 6 mo, 2 yrs)	Formation of a specific (putative I-compound) adduct in peripheral lung tissue DNA after 2 yrs of exposure.
Rehn et al. 2003	a.. P25 b. T805, 20 nm surface-silanised (Degussa)	rat	Intratracheal instillation (8-OHdG)	1.25 mg/rat	Immunohistochemistry of lung tissue did not reveal ROS-dependent 8-OHdG adduct formation 90 d p.e.
Trouiller et al. 2009	P25	mouse	Oral administration 1. In vivo DNA deletion transgenic mouse system 2. Alkaline comet assay (peripheral blood) 3. γ-H2AX assay (bone marrow cells) 4. Micronucleus assay) 5. 8-OHdG frequency (liver)	0-500 mg/kg (5d)	P25 induced 8-OHdG, micronuclei, DNA deletions, and γ-H2AX foci. Most assays positive at 500 mg/kg only. γ-H2AX foci increased dose-dependently. Systemic oxidative genotoxicity was assumed as underlying mechanism (but bioavailability of Ti not measured). No positive control.

Endpoint study record: Genetic toxicity in vivo_NM 105_COMET Instillation by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2013
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	NRCWE (DK)	**Report no.**	D6
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM105_MN Instillation

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Administration / exposure

Route of administration

intratracheal

Vehicle(s)

Rat Serum Albumin (RSA) 0.05% diluted (9:1 v/v) in 10x Phosphate buffer pH 7.4

Duration of treatment / exposure

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

Doses / concentrations

1.15, 2.3, 4.6 mg/kg bw/d

Basis nominal conc.

Control animals

yes

Positive control(s)

Methyl MethaneSulfonate 25 mg/kg bw/d

Examinations

Tissues and cell types examined

lung, BAL fluid, liver, spleen, kidney

Evaluation criteria

Median %Tail DNA from >100 cells per organ

Statistics

Kruskall wallis one-way test for negative vs treated

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D6_in vivo: **ENV/JM/MONO(2015)17/ANN10**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

TiO2 NM-105 is not genotoxic in rats at the tested doses following a short-term exposure via intratracheal instillation with the comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vivo_NM 105_MN Bone marrow Instillation by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2013
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	NRCWE (DK)	**Report no.**	D6
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM 105_COMET Instillation

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier according to

Guideline OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male

Administration / exposure

Route of administration

intratracheal

Vehicle(s)

Rat Serum Albumin (RSA) 0.05% diluted (9:1 v/v) in 10x Phosphate buffer pH 7.4

Duration of treatment / exposure

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

Doses / concentrations

1.15, 2.3, 4.6 mg/kg bw/d

Basis nominal conc.

No. of animals per sex per dose

5

Positive control(s)

Methyl MethaneSulfonate 25 mg/kg

Examinations

Tissues and cell types examined

Bone marrow

Evaluation criteria

2000 immature erythrocytes per rat

Statistics

Chi square test

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D6_in vivo: **ENV/JM/MONO(2015)17/ANN10**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

TiO2 NM-105 is not genotoxic in rats at the tested doses following a short-term exposure via intratracheal instillation with the micronucleus assay in bone marrow

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vivo_NM 105_COMET Gavage by NANOGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2013
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	IMB-BAS (BG)	**Report no.**	D6
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM105_MN Bone marrow Gavage

Materials and methods

Type of genotoxicity

DNA damage and/or repair

Type of study

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

Test guideline

Qualifier no guideline available

Guideline

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Test animals

Species

rat

Strain

Wistar

Sex

male

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

Rat Serum Albumin (RSA) 0.05% diluted (9:1 v/v) in 10x Phosphate buffer pH 7.4

Duration of treatment / exposure

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

Doses / concentrations

6.5, 13, 26 mg/kg bw/d

Basis nominal conc.

No. of animals per sex per dose

5

Control animals

yes

Positive control(s)

Methyl MethaneSulfonate 280 mg/kg bw/d

Examinations

Tissues and cell types examined

intestine, colon, blood, bone marrow, spleen, liver, kidney

Evaluation criteria

Median %Tail DNA from >100 cells per organ

Statistics

Mann–Whitney U test followed by Jonckheere-Terpstra trend test

Results and discussions

Additional information on results

Assay with FpG in Intestine and colon: Negative result

Overall remarks, attachments

Attached full study report

Attached document	D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**
Remarks	Dispersion protocol
Attached document	D6_in vivo: **ENV/JM/MONO(2015)17/ANN10**
Remarks	Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

other: Positive in colon: Dose-dependant increase in the % Tail intensity with the highest dose being significant Positive in spleen : Increase in the % Tail intensity at the highest dose Negative in intestine, blood,bone marrow, liver, kidney

Conclusions

TiO2 NM-105 is genotoxic in the colon and the spleen of rats following a short-term exposure via gavage with the alkaline comet assay.

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

Endpoint study record: Genetic toxicity in vivo_NM 105_MN Bone marrow Gavage by NANGENOTOX

Administrative Data

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

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

Data source

Reference

Reference type	study report		
Author	V Fessard	**Year**	2013
Title	Deliverable 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis		
Bibliographic source			
Testing laboratory	IMB-BAS (BG)	**Report no.**	D6
Owner company			
Company study no.		**Report date**	

Data access

other: Owner: NANOGENOTOX

Data protection claimed

yes, but willing to share

Cross-reference to same study

NM105_COMET Gavage

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier according to

Guideline OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)

Deviations

Test materials

Reference Material/Nanomaterial and Sample identification number

Identifier Reference Material/Nanomaterial

Identity NM 105

Test animals

Species

rat

Strain

Wistar

Sex

male

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

Rat Serum Albumin (RSA) 0.05% diluted (9:1 v/v) in 10x Phosphate buffer pH 7.4

Duration of treatment / exposure

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

Doses / concentrations

26 mg/kg bw/d

Basis **nominal conc.**

No. of animals per sex per dose

5

Positive control(s)

Methyl MethaneSulfonate 280 mg/kg

Examinations

Tissues and cell types examined

Bone marrow

Evaluation criteria

2000 immature erythrocytes per rat

Statistics

Chi square test

Overall remarks, attachments

Attached full study report

Attached document D2_WP4_ SOPs report: **ENV/JM/MONO(2015)17/ANN1**

Remarks Dispersion protocol

Attached document D6_in vivo: **ENV/JM/MONO(2015)17/ANN10**

Remarks Data in the report and detailes porocol in annex

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

TiO2 NM-105 is not genotoxic in rats at the tested dose following a short-term exposure via gavage with the micronucleus assay in bone marrow

Cross-reference to other study

http://www.nanogenotox.eu/: **ENV/JM/MONO(2015)17/ANN22**

7.6.3 Photogenotoxicity

Summary of the HH literature data status 03rd April 2014 : Summary of studies on Photogenotoxicity

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Photogenotoxicity					
Linnainmaa et al. 1997	1. P25 2. UV Titan M160 (20 nm, rutile, coated) 3. Pigmentary TiO_2 (anatase, 170 nm, Kemira)	RLE - Liver immortal epithelial cells (rat)	CBMN	5, 10 and 20 µg/cm^2 (20h)	Negative for single or combined treatment, irrespective of absence or presence of UV irradiation (366 nm, 5 min).
Nakagawa et al. 1997	P25	1. and 2. L5178Y lymphoma cells (mouse) 3. CHL/IU (hamster) 4. S. typhi-murium	1. Alkaline comet assay (SCG assay) 2. Mammalian cell mutation assay 3. Chromosome aberration assay 4. Ames test	1. 3.1 - 800 µg/ml (24h) 2. 250 – 2000 µg/ml 3. 0.78 – 50 µg/ml (-UV/vis) and 25 – 800 µg/ml (+UV/vis) (24h) 3. 5000 - 40000 µg/ml (48h)	No or only weak genotoxicity in the dark but significant chromosome aberrations upon UV/vis irradiation following industry standard protocol. The mutagenic assays were negative, indicating that photogenotoxic potential is rather clastogenic.
Theogaraj et al. 2007	P25 (?) plus 7 other different TiO_2 particle types of different shape, modification and crystallinity	CHO cells (hamster)	Chromosome aberration assay	800 – 5000 µg/ml (3h + 17 h recovery, - S9 mix)	UV-irradiated cells (750mJ/cm^2) showed no photogenotoxicity with either particle type at any concentration.

Gerloff et al. 2009	a. P25 b. Anatase, modified, < 10 nm (Sigma) c. Anatase, 40-300 nm (Aldrich)	Caco-2 colon adenocarcinoma cell line (human)	Fpg-comet assay	20 µg/cm^2	Increased DNA damage as well as significant oxidative damage only upon ambient light irradiation. No relationship between particle surface area and DNA damage.

7.7 Carcinogenicity

Summary of the HH literature data status 03rd April 2014 : Summary of studies on carcinogenicity

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Heinrich et al., 1995	P25	rat mouse		7.2 mg/m^3 for 4 months, 14.8 mg/m^3 for 8 mo, 9.4 mg/m^3 for 16 (5.5) mo.	Rats but not mice showed increased P25-induced tumor incidence after 18/24 months and accumulating pulmonary overload. Histopathology revealed a spectrum of benign and malignant (squamous cell carcinomas and adenocarcinomas) tumors.
Pott and Roller 2005	1. P25 2. Anatase, 0.2 µm (Sigma)	rat	Multiple intratracheal instillations	1. 5 x 3 mg 2. 5 x 6 mg 3. 10 x 6 mg (each regime over a period of 30 mo)	Tumor incidences for P25 (1. 52%, 2. 67%, 3. 70%) were significantly higher than for micron-sized TiO_2. Rapid development of broad spectrum of benign and malignant tumors at instant overload conditions. Authors postulated a linear dose-response relationship.

7.8 Toxicity to reproduction

7.8.1 Toxicity to reproduction

7.8.2 Developmental toxicity / teratogenicity

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Developm ent					
Scuri et al. 2010	P25	rat	Inhalation	12 mg/m^3; 5.6 h/d for 3 d	Age-dependent upregulation in the expression of lung neurotrophins associated with increased airway responsiveness in neonates and weanlings but not in adults.

7.9 Specific investigations

7.9.1 Neurotoxicity

Summary of the HH literature data status 03rd April 2014 : Summary of studies on neurotoxicity

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Wang et al. 2007c	a. rutile, 25 nm (Dayang) b. rutile, 80 nm (Dayang) c. fine anatase, 155 nm (Zhonglian)	mouse	Nasal inhalation (instillation?)	10 µl TiO_2 suspension (0.1 g/ml) every 2 d for 1 mo.	Microbeam SRXRF mapping techniques revealed that TiO_2 particles of different size and crystallinity translocated to the olfactory bulb via primary olfactory neurons, fine-sized particles showing wider distribution. The presence of particles affected the micro-distribution of Fe, Cu, and Zn in the olfactory bulb.
Shin et al. 2010	a. P25 b. Rutile, 1 µm (Sigma)	mouse	Intraperitoneal injection	1 mg/mouse (Pre-treament with 5mg/kg LPS, i.p.)	Elevated expression of proinflammatory cytokines (IL-1beta, TNF-alpha), increased ROS levels and activated microglia 24 h after LPS challenge in TiO_2-treated mouse brains. P25 had no effect w/o LPS pre-treatment. The cytokine response was lower for fine TiO_2.

7.9.2 Immunotoxicity

Summary of the HH literature data status 03rd April 2014 : Summary of studies on sensitization and immunotoxicity data

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Park et al. 2009	P25	mouse	Intratracheal Instillation	5, 20, 50 mg/kg	Dose-dependent induction of proinflammatory as well as Th1-type and Th2-type cytokine. B-cell proliferation in spleen and blood and increased IgE in BALF. Granuloma formation and expression of MIP-2 and MCP-1 in alveolar tissue. Microarray analysis revealed increased expression of MHC-1 class genes. It was concluded that P25 induced chronic inflammation in mice
Gustafsson et al. 2011	P25	Rat (Dark Agouti)	Intratracheal instillation	1, 5, and 7.5 mg/kg	Induction of long-lasting lymphocyte response after a transient innate immune activation of eosinophils, neutrophils, dendritic cells, and NK cells

7.9.3 Specific investigations: other studies

Summary of the HH literature data status 03rd April 2014 : Summary of studies on cardiovascular toxicity

Reference	Material/ Size	Test Organism/ System	Method	Exposure/ dose	Main findings
Nurkiewicz et al., 2008	a. P25 b. 1 µm (Sigma)	rat	Short-term inhalation	1.5 – 16 mg/m^3 (240 – 720 min)	Intavital analysis of the exteriorized spinotrapezius muscle together with functional tests and histopathology revealed that brief inhalative exposure to sub-inflammatory doses of P25 produced dose-dependent effects on vasodilation of systemic arterioles. These were more pronounced than with fine particles at equivalent pulmonary loads.
Nurkiewicz et al., 2009	a. P25 b. <5 µm (Sigma)	rat	Short-term inhalation	1.5 – 16 mg/m^3 (240 – 720 min)	Nanoparticle exposure at doses which induce microvascular dysfunction also decreases NO bioavailability by at least two functionally distinct mechanisms. The mechanisms involved appeared to be similar between fine- and nanosized particles but the effective dose for nanoparticles was more than six times lower.
LeBlanc et	P25	rat	Short-term	6 mg/m^3	P25 inhalation also interfered with

al. 2009			inhalation	for 240 min	coronary microvascular function: spontaneous tone was increased and endothelium-dependent vasodilation in subepicardial arterioles was impaired, likely involving altered microvascular permeability.
LeBlanc et al. 2010	P25	rat	Short-term inhalation	6 mg/m^3 for 240 min	Impaired endothelium-dependent vasoreactivity in coronary arterioles induced by P25 exposure is associated with ROS increases in the microvascular wall and an altered prostanoid formation.

Phototoxicity

Reference	Material/ Size	Test Organism / System	Method	Exposure/ dose	Main findings
Cai et al. 1992	P25	HeLa cells Cervix carcinoma (human)	1. Cytotoxicity (Colony forming assay) 2. Antitumor effect in vivo	12–120 µg/ml UV irradiation: 10 min (500 W Hg lamp)	Dose-dependent cytotoxicity in the presence of UV-light, involving ROS that possibly interfere with mitochondrial electron transport chain. Photoexcited particles also suppressed the growth of HeLa cells implanted in nude mice
Sakai et al. 1994	P25	T24 Bladder tumor (human)	Cytotoxicity (Colony forming assay)	100 µg/ml UV irradiation: 4-6 min (150 W Xenon lamp)	Fluorescence indicator studies suggested that cell death induced by photo-excited P25 is associated with prior increase in cell membrane permeability for Ca^{2+}.
	a. P25 (Degussa, ~21 nm) b. anatase ("HR3", 5-10 nm; Zhejiang Hongsheng) c. rutile ("DJ3", 50 nm; Zhejiang Hongsheng)	1. cell-free 2. skin homogenates (mouse)	Western blotting	Reaction mixture: BSA (0.5 mg/ml), $NaNO_2$ (0.25–1.0 mM), and suspended TiO_2 (0.2–3.0 mg/ml) were mixed sufficiently in 0.1 M phosphate buffered saline (PBS) at pH 7.0. UV light illumination using a GYZ220-230V 250 W lamp (Philips) in a distance of 30 cm for 8 h.	P25 and anatase showed a high photocatalytic activity regarding to protein tyrosine phosphorylation, both in a reaction mixture and in skin homogenate, whereas rutile photocatalytic activity was low.
	22 nm anatase/rutile 25 nm anatase 31 nm anatase/rutile (P25) 59 nm	ARPE-19 retinal pigment epithelial cells (human)	Cell viability (PI) Flow cytometry (ROS: mitosox fluorescence) Thiobarbituric acid reactive	0, 0.3, 1, 3, 10, 30, or 100 µg/ml for 24 UVA: 2 hrs, 7.53 J/cm^2	UVA lowered cell viability and increased ROS generation. The 25 nm anatase and 31 nm anatase/rutile were the most phototoxic (LC_{50} with UVA < 5 µg/ml), while the 142 nm anatase and 214 nm rutile were the least phototoxic. Relative potency of the six samples:

	anatase/rutile 142 nm anatase 214 nm rutile		substance (TBARS) assay (nanoparticle reactivity)		smaller particles being more toxic; larger surface areas being more toxic; particles generating more ROS being more toxic.

Toxicity in vitro

Reference	Material/ Size	Test Organism / System	Method	Exposure/ dose	Main findings
Renwick et al. 2001	a. "UTiO$_2$", 29 nm (Degussa) (P25?) b. fine TiO$_2$, 250 nm (Tioxide)	J774.2,MΦ Alveolar macrophage cell line (mouse)	Phagocytic activity	0.0975– 0.78 µg/mm^2 for 8 h	Dose-dependent impairment of indicator bead phagocytosis. Nanoparticles had a slightly more inhibitory effect.
Xia et al. 2006	P25	RAW 264.7 macrophage cell line (mouse)	ROS production (fluorescence analysis)	10 µg/ml (4h-16h)	P25 was capable of ROS generation in a cell-free system but incapable of doing so in RAW 264.7 cells, despite effective particle uptake.
Kang et al. 2008a	a. P25 b. Fine TiO$_2$, 1 µm (Sigma)	RAW 264.7 macrophage cell line (mouse)	ROS production (fluorescence analysis)	0.5–100 µg/ml (4-24h)	P25 enhanced intracellular ROS generation to a greater extent than fine TiO$_2$. P25 induced ERK1/2 activation, and enhanced secretion of proinflammatory TNF-∝ and MIP-2 in a concentration-dependent manner.
Han et al. 2008	1. P25	L929 fibrocytes (mouse)	1. Cell proliferation (MTT assay) 2. Apoptosis (Propidium iodide)	50, 100, 200 µg/ml (24-72h)	P25 (as well as several other TiO$_2$ anatase or rutile nanoparticles of 20-100 nm) had no effect on cell proliferation or apoptosis.
Komatsu et al. 2008	Anatase TiO$_2$, 25-70 nm (Aldrich)	TM3 Leydig cell line (mouse)	Cell proliferation and function	100 µg/ml (24 h)	Inhibition of viability and a transiently reduced proliferation but no direct effect on the induction of oxidative stress or synthesis of testosterone.
Gerloff et al. 2009	P25	Caco-2 Colon adenocarcinoma cell line (human)	Cytotoxicity (LDH and WST-1 assay)	1.25, 5, 20 and 80 mg/cm^2 (4 and 24 h)	Dose-dependent cytotoxicity observed with LDH (disturbed membrane integrity) and WST-1 (disturbed metabolic activity).
VanWinkle et al. 2009	nano-TiO$_2$, ~ 25 nm (Degussa; P25?)	R3-1 Type I-like alveolar epithelial cells (rat)	1. Cellular uptake (TEM/EDS) 2. ROS generation (Amplex Red	1. 1.2 µg/cm^2 (24 /48h) 2. 0.4 µg/cm^2 (20 min)	Particle uptake and subsequent localisation in cytoplasm, mitochondria and lysosomes but no increased intracellular H_2O_2 production or ROS-induced cell death.

			assay)		
Zhao et al. 2009	a. P25 b. Rutile, > 5 µm (Sigma)	JB6 epidermal cells (mouse)	Apoptosis (MTT assay, YOPRO-1 iodide staining, Western blot analysis)	0.1-20 µg/cm^2 (24 h)	P25 more potent in inducing apoptosis than fine particles. Evidence was presented that the apoptotic pathway involved caspase-8/Bid and intrinsic mitochondrial pathways as well as mitochondrial membrane injury.
Barillet et al. 2010	P25	NRK-52E kidney cells (rat)	1. Cell viability (MTT and LDH assay) 2. ROS production (FDA fluorescence assay)	1. 0.25-100 µg/ml (48 h) 2. 50, 100, 200 µg/ml (24 h)	Dose-dependent increase of cell mortality and intracellular ROS generation.
Simon et al. 2010	P25 (fluorescent dye-modified and native)	Primary foreskin keratinocytes (human)	1. Cell proliferation 2. Cytotoxicity (apoptosis) 3. Intracellular Ca^{2+}-homeostasis (high-resolution imaging analysis)	1. 0.2, 2, 20 µg/cm^2 (8d) 2. 2 µg/cm^2 (24 h)	Internalization of P25 induced an increase in intracellular Ca^{2+}, as well as a dose-dependent decrease in cell proliferation, actin reorganization and keratinocyte differentiation, but no cytotoxicity. Fluorescence-labelled particles were less toxic than unlabelled particles.
Long et al. 2006	P25	BV2 microglia (mouse)	1. ROS production (luminescence probing) 2. Cell viability (intracellular ATP levels)	1. 2.5-120 ppm (5-120 min) 2. 2.5-120 ppm (6 and 18 h)	P25 induced a rapid, sustainable ROS release by oxidative burst and by interference with mitochondrial electron transport chain without seriously affecting cell viability.
Long et al. 2007	P25	BV2 microglia (mouse) N27 dopaminergic neurons (rat) Primary embryonic striatum cells (rat)	1. Microarray analysis 2.Cell viability (intracellular ATP levels) 3. Apoptosis	2.5–120 ppm (24-72h, depending on cell culture type)	

Liu et al. 2010	P25	PC12 pheochromocytoma nerve cells (rat)	1. Cell viability (MTT assay) 2. Intracellular ROS production (fluorometry 3. Apoptosis (PI flow cytometry)	1-100 µg/ml (6 - 48 h)	P25 elicited a dose- and time-dependent decrease in cell viability associated with intracellular accumulation of ROS and apoptosis.
Shin et al. 2010	P25	BV2 microglia (mouse)	TNF-alpha (ELISA) NF-kappaB binding (EMSA)	100ng/ml LPS or P25 alone or in combination for 24h	P25 induced ROS generation and activated LPS-stimulated microglia leading to enhanced TNF-alpha production and elevated NF-kappaB binding activity.
Liu et al 2010a	P25	Primary alveolar macrophages (rat)	Assays on Nitric oxide, cytokines, Neutral red uptake, chemotactic migration, F_c receptor-mediated rosette formation MHC II cytometry	18.75, 37.5, 75, 150, 300 µg/ml (24h)	TiO_2 induced inflammatory response (as indicated by NO and TNF-alpha level increases) and inhibited immune functions of alveolar macrophages, e. g. phagocytosis, chemoattraction, F_c- and MHC-receptor expression, TNF-alpha and NO synthesis.
Winter et al. 2010	a. P25 (PPD range: 20-80 nm; Evonik) b. DQ 12 (PPD range: 40-300 nm, Sigma Aldrich)	Bone marrow-derived dendritic cells (mouse)	Particle uptake, stimulation and inflammasome activation of dendritic cells in vitro; IL-1 beta determination in supernatant (ELISA) and apoptosis (annexin V staining)	1. 5–50 µg/cm2 for18 h for stimulation. 2. 20 or 40 µg/cm2 (2h) for inflammasome activation after 6h pre-stimulation with LPS (0.1 µg/ml) ± incubation with cytochalasin D (1.5 µg/ml, 30 min.) to block actin polymerization after pre-stimulation with LPS.	P25 induced upregulation of MHC-II, CD80, and CD86 on dendritic cells, and activated the inflammasome, leading to significant secretion of IL-1beta-in wild-type but not Caspase-1- or NLRP3-deficient mice. P25 also led to enhanced ROS production.

7.10 Exposure related observations in humans

7.10.1 Health surveillance data

7.10.2 Epidemiological data

7.10.3 Direct observations: clinical cases, poisoning incidents and other

7.10.4 Sensitisation data (humans)

7.10.5 Exposure related observations in humans: other data

Endpoint study record: Exposure related observations in humans: other data.001

Administrative Data

[IP] OECD: HPVC
Purpose flag key study ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability 2e -study well documented, meets generally accepted scientific principles, acceptable for assessment

Data source

Reference

Reference type study report
Year 2009
Title Exposure assessment of workplaces manufacturing nanosized TiO2 and silver
Bibliographic source Inhalation Toxicology

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Endpoint addressed

not applicable

Test guideline

Qualifier according to
Guideline other guideline:
Deviations no data

GLP compliance

yes

Test materials

Test material identity
Identifier CAS number
Identity 5949-29-1

Details on test material

- Name of test material (as cited in study report): TiO2 nanoparticles - Substance type: no data - Physical state: no data - Analytical purity: 99.0 ~ 102.0 % - Impurities (identity and concentrations):no data - Composition of test material, percentage of components:no data - Isomers composition:no data - Purity test date:no data - Lot/batch No.:no data - Expiration date of the lot/batch:no data - Stability under test conditions:no data - Storage condition of test material:no data - Other:no data

Method

Ethical approval
confirmed, but no further information available

Exposure assessment

measured

Overall remarks, attachments

Attached background material

Table 1. Information on workplaces.docx

Table 1. Information on workplaces

Plant	Region (handling workers)	Manufactured materials	Process	Engineering controls	PPE use
A (Research Institute)	Seoul (1)	TiO2 manufacturing	Chemical synthesis (pilot test)	Fume hood	Half-mask, Lab coat, glove
B (Research Institute)	Seoul (2)	TiO2 manufacturing	Chemical synthesis (Mass production)	Enclosure local exhaust system, glove box	Working clothes

fig 1.docx

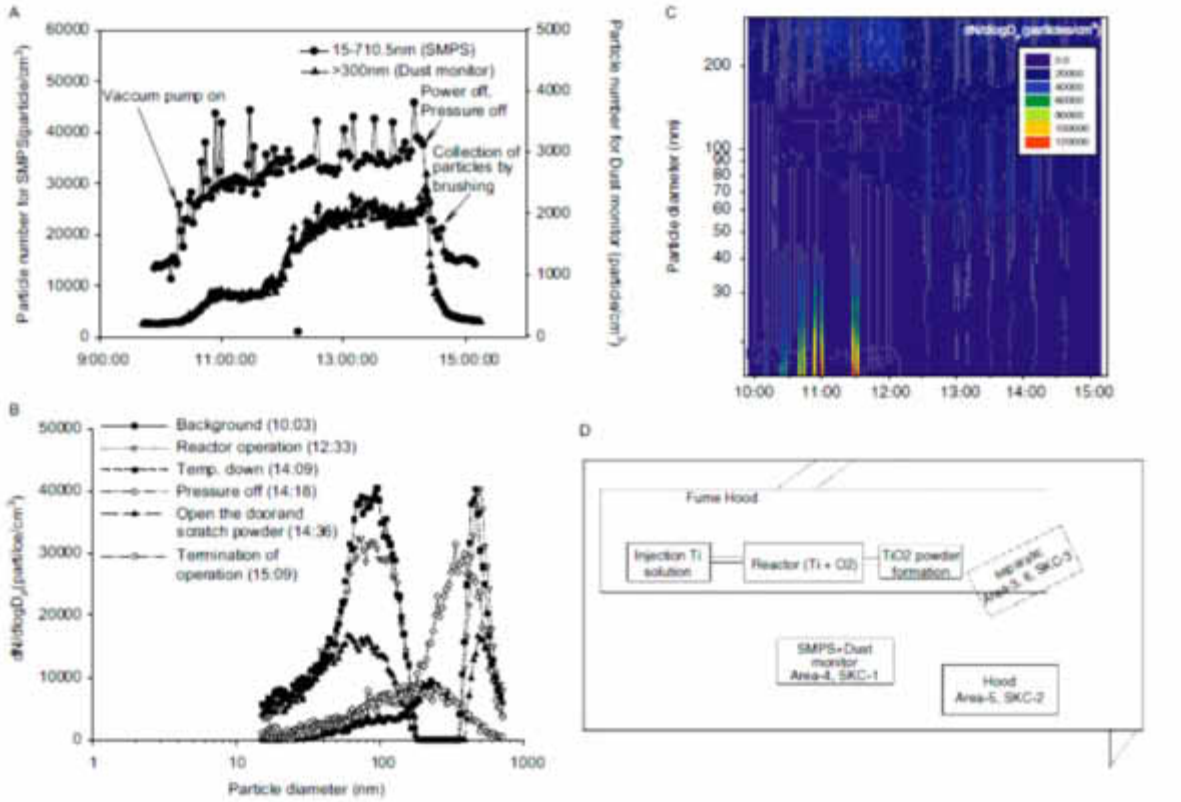


Figure 1. Real-time particle measurement in workplace A. (A) Particle number concentration measured by scanning mobility particle sizer (SMPS) and dust monitor; (B) particle size distributions measured by SMPS; (C) change in the particle size distributions by SMPS, and (D) process and sampling locations.

table 2.docx

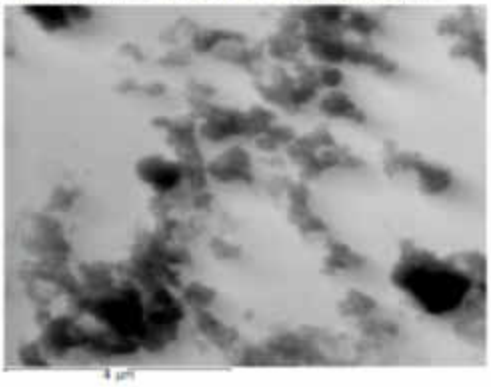
Table 2. TiO_2 mass concentration of personal and area samples at workplaces A and B.

Workplace	Process	Sampling site	Sampling time (min)	Sampling volume (L)	Mass concentration (mg/m³)*
Workplace A	During reactor operation	Area 4	298	595.4	0.50386
		SKC 1	185	1299.5	0.33090
		Area 5	296	585.2	0.63227
		SKC 2	292	2051	0.11702
	During powder collection	Area 3	18	36.1	4.99251
		Area 6	20	39.7	3.27456
		SKC 3 (personal)	30.9	216.6	0.55402
Workplace B	During operation	Area 3	350	701.1	0.29955
		SKC 3	317	2222	0.15752
		Area 6	322	630.8	0.15853
		SKC 2	315	2210	0.09502
		Area 4	263	525.5	0.89443
		Cyclone 8	312	754.7	0.30475
		Cyclone 7	313	756.8	0.18498
	Only during collection	SKC 1	47.8	334.6	0.47818

*Total suspended particulate concentrations were reported.

fig 2.docx

A. Transmission electron micrograph of TiO_2 nanoparticles



B. Energy dispersive x-ray profile (TiO nanoparticles on copper grid).

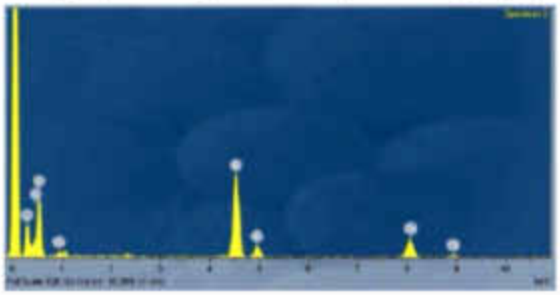


Figure 2. Transmission electron micrograph of TiO_2 nanoparticles sampled on the area 4 filter (Figure 1D). (A) Transmission electron micrograph of TiO_2 nanoparticles. (B) Energy dispersive X-ray profile (TiO_2 nanoparticles on copper grid).

fig 3.docx

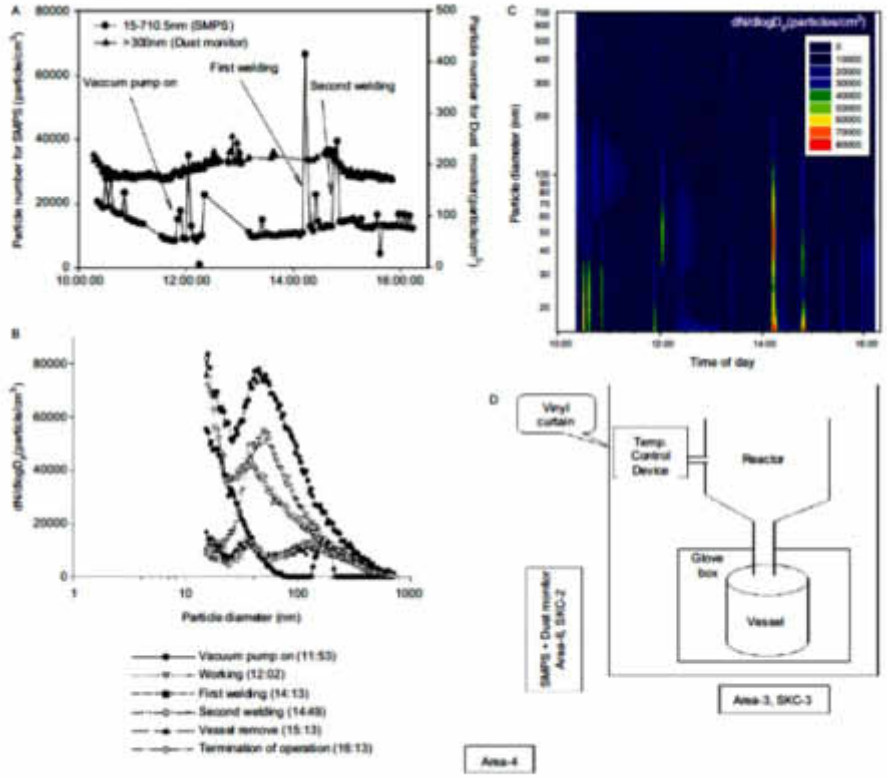


Figure 3. Real-time particle measurement in workplace B. (A) Particle number concentration measured by scanning mobility particle sizer (SMPS) and dust monitor; (B) particle size distributions measured by SMPS; (C) change in the particle size distributions by SMPS; and (D) process and sampling locations.

Applicant's summary and conclusion

Conclusions

The gravimetric concentrations of TiO2 ranged from 0.10 to 4.99 mg/m^3, which were lower than the occupational exposure limit 10 mg/m^3 set by the Korean Ministry of Labor or ACGIH (American Conference of Industrial Hygienists). The particle number concentrations at the nano TiO2 manufacturing workplaces ranged from 11,418 to 45,889 particles/cm^3 with a size range of 15 - 710.5 nm during the reaction, while the concentration decreased to 14,000 particles/cm^3 when the reaction was stopped. The particle concentrations at the TiO2 manufacturing workplaces increased during the reactor and vacuum pump operations, and during the collection of the synthesized TiO2 particles. The TiO2 nanoparticle concentrations were relatively lower than existing occupational exposure limits.

7.11 Toxic effects on livestock and pets

7.12 Additional toxicological information

7.13 In vitro toxicological information

Endpoint study record: cyto-toxicity by INIA

Administrative Data

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

Data source

Data access

other: performed and provided by INIA, Spain

Materials and methods

Type of information

An array of in vitro cytotoxicity assays were performed in a mammalian (H4IIE) and fish cell line (RTG-2) following exposure to NM 105, P-25 EVONIK TiO2 nanoparticles.

Principles of method if other than guideline

(MTT) Methyl thiazol tetrazolium salt reduction assay (NRU) Neutral red uptake assay (LDH) Lactate dehydrogenase

Describe the scientific and technical basis of the test method

What biological/cellular model is the method based on?

Rat hepatoma cell line (H4IIE) and Rainbow trout (Oncorhyncus mykiss) gonadal cell line (RTG 2)

What biological endpoints/responses does this method address?

cyto-toxicity

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

Sample administration 96-well cell culture plate Exposure route : Directly on cells Exposure duration 24 h and 72 h Concentration tested 100-0.003 µg/mL

Performance assessment of the method

Test materials

Details on test material

NM 105 P-25 EVONIK

Sample preparation/conditioning protocol

Appropriate amount of TiO2 particles in a 20 ml vial. Particles were transferred to exposure medium and vial washed thoroughly. Suspension were stirred overnight at approx. 900 rpm with a magnetic stirrer. Exposure dilutions were made during stirring. Unused suspensions were kept in closed bottles wrapped in aluminium foil and stirred for 1 h before reusing.

Method

Any other information on materials and methods incl. tables

Particle exposures: In a 96 well-plate, 100 µl of cell suspension at 2.5×105 cells ml−1 for the H4IIE cell line and 1×105 cells ml−1 for the RTG-2 cell line were added to each well and then incubated for 24 h at 37 °C and 5% CO2 for the H4IIE or 20°C and 5% CO2 for the RTG-2 cell line respectively. After 24 h, cells were exposed to the respective TiO2 nanoparticles with the highest nominal exposure concentration of 100 µg / mL. The remaining concentrations, ranging from 50 µg / mL to 0.003 µg /mL were produced by a 50 % downwards dilution for all assays. All treatments were done in triplicate. A positive control containing sodium dodecyl sulfate (SDS) was also included, ranging from 10 µg /mL to 0.3 µg /mL. The exposure duration was 24 h or 72 h. For 72 h exposures the cell media was renewed prior to exposure. Cytotoxicity assays: MTT assay Cell viability was determined via the ability of living cells to reduce the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) to formazan as described by (Mosmann, 1983). The cells were washed with PBS after the exposure to the respective particle and 100 µL of EMEM media without phenolred containing 0.5 mg /mL of MTT was added in each well. The plates were then incubated at the appropriate temperature and 5% CO2 for 4 h. The precipitated blue formazan product was then extracted using isopropanol as a solvent. The optical density (OD) of each well was determined at a wavelength of 570 nm using a plate reader (Tecan GENios, Madrid, Spain). The data was then expressed as a percentage of the control. Neutral red uptake assay (NRU) Cell viability was determined via the ability of intact lysosomes to retain the dye neutral red in living cells as described by (Borenfreund and Puerner, 1985). The cells were washed with PBS after the exposure to the respective particle and 100 µL of EMEM media without phenolred containing 0.05 mg /mL of neutral red was added in each well. The plates were then incubated at the appropriate temperature and 5% CO2 for 4 h. After incubation the cells were washed again with PBS and the neutral red retained by the cells was extracted using 100 µL of 1% acetic acid in 50% ethanol per well. The optical density (OD) of each well was determined at a wavelength of 550 nm using a plate reader (Tecan GENios, Madrid, Spain). The data was then expressed as a percentage of the control. The lactate dehydrogenase (LDH) assay The concentration of LDH in cells or released to the medium was determined as described by Brown et al., 2001. The assay is based on the ability of LDH to catalyze the conversion of pyruvate to lactate with simultaneous conversion of NADH to NAD+. The pyruvate not converted by LDH attaches to 2,4-dinitrophenylhydrazine and forms a brown complex. The intensity and therefore the measured absorption is inversely proportional to the LDH concentration. Triton X-100 is used as a positive control since its assumed to cause 100 % cell death and therefore represents the total releasable LDH.

Results and discussions

Remarks on results including tables and figures

The assays were conducted with H4IIE and RTG 2 cell lines with various concentrations and 24 h or 72 h as exposure durations. The MTT, NRU as well as the LDH assay, for assessing cytotoxicity, showed no negative effect due to exposure to the tested Titanium dioxide particles at the concentrations used (100 mg/L – 0,003 mg/L).

Applicant's summary and conclusion

Cross-reference to other study

MOSMANN, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 65, 55-63. BORENFREUND, E. & PUERNER, J. A. (1985) Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicol Lett, 24, 119-24. BROWN, D. M., WILSON, M. R., MACNEE, W., STONE, V. & DONALDSON, K. (2001) Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol Appl Pharmacol, 175, 191-9.

Endpoint study record: cyto-toxicity by University of Alberta

Administrative Data

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

Data source

Data access

other: performed and provided by University of Alberta, Canada

Cross-reference to same study

"In vitro cytotoxicity and genotoxicity studies of titanium dioxide (TiO2) nanoparticles in Chinese hamster lung fibroblast cells" Mahsa Hamzeh, Geoffrey I. Sunahara Toxicology in Vitro 27 (2013) 864–873 Monitoring of potential cytotoxic effects of titanium dioxide using on-line and non-invasive cell-based impedance spectroscopy. K. B. Male, M. Hamzeh, J. Montes, A. C.W. Leung and J. H.T. Luong. (submitted, Analyst, 2012)

Materials and methods

Type of information

inhibitory/cytotoxic effects

Principles of method if other than guideline

electric cell substrate impedance sensing: Cells attach to a gold electrode surface pre-coated with a protein (fibronectin for mammalian cells and Concanavalin A for insect cells) recognized by the cell line. The test substance is added simultaneously with the cell line chosen and the resistance change is monitored with time and then an ECIS50 value is determined based on inhibitory/cytotoxic effects. The effect of UV light exposure of titanium dioxide nanoparticles was also evaluated by the addition of a UV light source to the incubation chamber of the ECIS system. The methodology has been referenced in the literature numerous times by the Luong group.

Describe the scientific and technical basis of the test method

What biological/cellular model is the method based on?

Chinese hamster lung fibroblast V79 cells (mammalian) 1.5 x 10^6 cells/mL Spodoptera frugiperda Sf9 (insect cells) 3 x 10^6 cells/mL

What biological endpoints/responses does this method address?

inhibitory/cytotoxic effects

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

Sample (titanium dioxide) was added as a suspension in culture medium (SF-900 II for insect cells and DMEM for mammalian cells) along with cell in culture medium to the wells of the ECIS plates. The exposure duration is up to 24 hours. The concentrations of titanium dioxide nanoparticles tested were in the range of 30-400 ppm. ECIS50 values were determined as SEM (standard error on the mean) for n=3. For each experiment, 6 concentrations of the test substance were run in duplicate. Each test substance was then evaluated 3 times. Controls are run as follows: The system (wells with protein coated gold electrodes) is pre-run with culture medium for 1 hour to establish a flat baseline. Controls (4 of 16 well positions for each run) containing cells without the test substance (titanium dioxide) are run with each experiment. The controls are critical in order to calculate the ECIS50 values. For each experiment, 6 concentrations of the test substance were run in duplicate. Each test substance was then evaluated 3 times. Controls are run as follows: The system (wells with protein coated gold electrodes) is pre-run with culture medium for 1 hour to establish a flat baseline. Controls (4 of 16 well positions for each run) containing cells without the test substance (titanium dioxide) are run with each experiment. The controls are critical in order to calculate the ECIS50 values. Ethanol at 2 % is added to the culture medium for trials with insect cells.

Describe the Standard Operating Procedure (SOP)

SOP description as a template

No significant protocol deviation from methods reported by the Luong group in the literature.

Performance assessment of the method

Test materials

Details on vehicle/medial matrix

For mammalian cells (V79) the medium used was DMEM and for insect cells (Sf9) the medium used was SF-900 II. Ethanol at 2 % is added to the culture medium for trials with insect cells.

Sample preparation/conditioning protocol

Dispersion of TiO2 in medium. Sample is dispersed by homogenization (stock solutions in the range of 400-800 ppm in culture medium) at 5000 rpm for 5 min using a IKA® T25 digital Ultra-Turrax homogenizer (IKA® Works Inc., Wilmington, NC, USA). Samples were used the same day of homogenization and vortexed before use to have as homogeneous solution as possible, since the sample will sediment out with time.

Method

Any other information on materials and methods incl. tables

No significant protocol deviation from methods reported by the Luong group in the literature.

Results and discussions

Remarks on results including tables and figures

Titanium dioxide nanoparticles did not displayed very significant cytotoxic effects with respect to mammalian cells (Chinese hamster lung fibroblast V79 cells) using electric cell-substrate impedance sensing (ECIS) as a screening technique. Significant cytotoxic effects were not observed up to 400 ppm with P25. Using insect cells (Spodoptera frugiperda Sf9) as an ECIS test model, inhibitory effects were observed for all TiO2 samples. P-25 exhibited ECIS50 values of 211 +/- 6 ppm. Exposure of titanium nanoparticles to UV light at either 254 nm or 365 nm had no significant effect on the ECIS50 value.

Overall remarks, attachments

Overall remarks

ECIS results demonstrated that nano titanium dioxide did not display significant cytotoxic effects on either mammalian or insect cells attached to protein coated gold electrodes. Observation of the electrode surface after 24 h indicated the presence of many viable mammalian cells (not many dead cells were observed as is the case with other potent toxins) still attached to the electrode surface, however the cells were not as well spread as in the case of the control (no titanium dioxide nanoparticles). Insect cells on the surface look more spherical compared to the flattened forms observed with the controls. Inhibitory effects were noted at the substratum level (adhesion and spreading of cells on the gold surface) resulting in differential changes in the resistance response which were dose dependent. The ECIS50 values varied for different nano titanium dioxide test substances, which is likely due to their differences with respect to factors such as: particle size, charge (zeta potential), particle surface area and composition (anatase vs. rutile). Exposure of the titanium dioxide nanoparticles to UV light did not alter the ECIS50 values.

Attached full study report
Hamzeh_Sunahara_ in vitro cytotox genotox.pdf: ENV/JM/MONO(2015)17/PART1/ANN24

Applicant's summary and conclusion

Conclusions

Cell-based impedance spectroscopy measurement in combination with Sf9 cells and V79 fibroblast cells is a simple and reliable tool for screening potentially cytotoxic/inhibitory effects of nanoscale materials. ECIS results demonstrated that titanium dioxide nanoparticles did not display significant cytotoxic effects on either mammalian or insect cells attached to the protein coated gold electrodes. This noninvasive and real-time approach allows quantitative assessment of biocompatibility of lead nanoscale materials such as titanium dioxide nanoparticles for diversified applications.

Cross-reference to other study

J.H.T. Luong, M. Habibi-Razaei, J. Meghrous, C. Xiao, K.B. Male, and A. Kamen, Anal. Chem., 2001, 73, 1844-1848. C. Xiao, B. Lachance, G. Sunahara, and J.H.T. Luong, Anal. Chem., 2002, 74, 5748-5753. C. Xiao, and J.H.T. Luong, Biotechnol. Prog., 2003, 19, 1000-1005. K.A. Mahmoud., J. Mena, K.B. Male, S. Hrapovic, A. Kamen, and J.H.T. Luong, Appl. Mater. & Interfaces, 2010, 2, 2924-2932. K.B. Male, B. Lachance, S. Hrapovic, G. Sunahara, and J.H.T. Luong, Anal. Chem. 2008, 80, 5487-5493. K.B. Male, Y.K. Rao, Y.-M. Tzeng, J. Montes, A. Kamen, and J.H.T. Luong, Chem. Res. Toxicology. 2008, 21, 2127-2133. K.B. Male, Y-M. Tzeng, J. Montes, B-L. Liu, W-C. Liao, A. Kamen, and J.H.T. Luong, Analyst 2009, 134, 1447-1452. K.B. Male, R. Tom, Y. Durocher, C. Greer, and J.H.T. Luong. Environmental Science and Technology. 2010 44, 6775-6781. K. B.Male, A.C.W. Leung, J. Montes, A. Kamen, and J.H.T. Luong, Nanoscale, 2012, 4, 1373-1379. K.B. Male, E. Lam, J. Montes, and J.H.T. Luong, Appl. Mater. & Interfaces, 2012, 4, 3643-3649.

Endpoint study record: In vitro toxicological information byUS EPA

Administrative Data

Purpose flag key study ( ) robust study summary ( ) used for classification ( ) used for MSDS
Study result type experimental result
Reliability 1 (reliable without restriction)
Rationale for reliability Peer Reviewed Publication

Materials and methods

Type of information

For in vitro treatment, particles were suspended in cell culture media containing 10% FBS (Allouni et al., 2009). A stock suspension of 1 mg/ml was prepared and then dispersed using a probe sonicator (Misonix Microson Ultrasonic Cell Disrupter XL, Farmingdale, NY) operating at 4.5 watts for 3 pulses, 2 seconds/pulse. This sonication protocol consistently dispersed the nanoparticles until the suspension appeared homogenous to the naked eye. Other concentrations were prepared by serially diluting the stock suspension in the same media. A newstock suspension wasmade from dry nano-TiO2 immediately before treating cells in each experiment. The suspensions were relatively stable over time (for at least 20 minutes according to dynamic light scattering) and could readily be evaluated and applied to the cells to be treated, as reported by others (Allouni et al., 2009). Cells were treated with 0, 0.3, 1, 3, 10, 30, or 100 µg/ml nano-TiO2 in media for 24 hrs and then exposed to UVA (2 hrs, 7.53 J/cm2) or kept in the dark. Viability was assessed 24 hrs after the end of UVA exposure by microscopy with a live/dead assay (calcein-AM/propidium iodide).

Describe the scientific and technical basis of the test method

What biological/cellular model is the method based on?

A human-derived RPE cell line (ARPE-19) was obtained from ATCC (Manassas, Virginia) and grown in a 1:1 combination of Dulbecco's Modified Eagle's Medium and Ham's F-12 Nutrient Mixture (DMEM/F-12; cat # 11039047, Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS; cat # 511150, Atlanta Biologicals, Lawrenceville, GA) and 1% penicillin/streptomycin (cat # 17-602E, Lonza,Walkersville, MD). Phenol red was omitted from the media due to its potential interaction with UV radiation.

Overall remarks, attachments

Overall remarks

Exposure to higher concentrations of nano-TiO2 with UVA lowered cell viability. The 25 nm anatase and 31 nm anatase/rutile were the most phototoxic (LC50 with UVAb5 µg/ml), while the 142 nm anatase and 214 nm rutile were the least phototoxic. An acellular assay ranked TiO2 nanoparticles for their UVA photocatalytic reactivities. The particles were found to be capable of generating thiobarbituric acid reactive substances (TBARS) under UVA. Flow cytometry showed that nano-TiO2 combined with UVA decreased cell viability and increased the generation of reactive oxygen species (ROS, measured by Mitosox). LC50 values under UVA were correlated with TBARS reactivity, particle size, and surface area.

Endpoint study record: buccal mucosa permeability by University of Graz

Administrative Data

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

Data source

Data access

other: performed and provided by University of Graz, Austria

Cross-reference to same study

Roblegg E., Fröhlich E., Samberger C., Zaversky M., Teubl B., Zimmer A., Evaluation of a physiological in-vitro system to study the transport of nanoparticles through the buccal mucosa, Nanotoxicology. 2011 May 18. [Epub ahead of print] PMID: 21591874 Teubl B., Meindl C., Eitzelmayer A., Zimmer A., Fröhlich E. and Roblegg E., In-vitro Permeability Studies of neutrally charged Polystyrene Particles through the Buccal Mucosa, submitted 2012 Teubl B., Leitinger G., Fröhlich E., Schneider M., Tockner M., Zimmer A., Roblegg E., Buccal Mucosa as a Route for TiO2 Uptake, in preparation

Materials and methods

Principles of method if other than guideline

Method/ guideline followed: Roblegg E., Fröhlich E., Samberger C., Zaversky M., Teubl B., Zimmer A., Evaluation of a physiological in-vitro system to study the transport of nanoparticles through the buccal mucosa, Nanotoxicology (2012) 6, 399-413 Teubl B., Meindl C., Eitzelmayer A., Zimmer A., Fröhlich E. and Roblegg E., In-vitro Permeability Studies of neutral Polystyrene Particles through the Buccal Mucosa, small 2012, DOI: 10.1002/smll.201201789

Describe the scientific and technical basis of the test method

What biological/cellular model is the method based on?

excised porcine buccal mucosa

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

Permeability studies: • Exposure route: oral/buccal • Exposure duration: 4 h • Concentration tested: 100 µg/ml PBS (pH 7.4) • Description of the method and give justification:, "no guidelines available" Porcine mucosa is the most similar one to the human mucosa in ultra structure as well as in enzyme activity and was obtained from freshly sacrificed pigs (age: < 6 months; Karneta Slaughter House, Graz, Austria). Ten minutes after slaughtering, the mucosa was immediately stored in 4°C Krebs buffer (KB), transferred to the laboratory and used within 1 hour post mortem. The underlying tissue was removed with a scalpel blade and carefully trimmed with surgical scissors to achieve uniform thickness. During preparation the tissue was rinsed with 4°C KB every two minutes to prevent dehydration of the tissue. Prior to every experiment, MTT-tests were carried out to assure the viability of the tissue. As negative control, samples were boiled in water for one hour to deactivate the tissue (zero value). The measured values were calculated as absorbance units per mg tissue (i.e., tetrazolinium reductase index (TR Index)). Additionally, the integrity of the tissue was checked. The integrity test of the membrane was carried out using methylene blue/ PBS (1mg/ml) and methylene blue/ EDTA/ PBS (1mg/0.5mM/ml).The oral barrier studies were performed with static Franz diffusion cells (PermeGear, USA, 11.28 mm jacketed cell with a flat ground (ground o-ring) joint and clear glass with an 8 ml receptor volume). Each cell consisted of a

donor and of a receiver compartment. The receiver compartment was surrounded by a water jacket to assure a physiological temperature of 37 ± 0.5 °C throughout the experiment. The receiver compartment was filled with 7.8 ml PBS buffer and heated to 37 °C before use. A magnetic stirrer was used with an agitation of 300 rpm to assure equal distribution. Between the compartments the excised viable and integral sheet of mucosa was inserted and fixed with retainer clips in such a way that the epithelium faces the donor and the connective tissue region faces the receiver compartment. After an equilibration time of 30 min, the buffer in the donor compartment was replaced by TiO2 particles dispersed in PBS in a concentration of 100 µg/ml. After 4 h test duration, the mucosa was washed 3 times with PBS, fixed and embedded. Observation of the tissue samples was carried out by transmission electron microscopy and the particles were verified by element-analyses. • Analytics (analytical verification) The penetration behavior of NM100 particles was evaluated by Transmission Electron Microscopy (TEM). The tissue was fixed with 0.1M sodium phosphate buffered 2.5% glutaraldehyde overnight at 4°C and post-fixed in 1.0% osmium tetroxide. Dehydration was carried out through a graded series of ethanol to 100%. Subsequently, the tissue was transferred into propylene oxide and embedded into epoxy resin. Thin tissue sections were cut with a diamond knife and placed onto 300 mesh copper grids. The grids were not stained with heavy metals to prevent staining precipitates. Transmission Electron Microscopy images were obtained using a TEM model Tecnai equipped with an energy filter. Permeability studies: • Exposure route: oral/buccal • Exposure duration: 4 h• Concentration tested: 100 µg/ml PBS (pH 7.4) • Description of the method and give justification:, "no guidelines available" Porcine mucosa is the most similar one to the human mucosa in ultra structure as well as in enzyme activity and was obtained from freshly sacrificed pigs (age: < 6 months; Karneta Slaughter House, Graz, Austria). Ten minutes after slaughtering, the mucosa was immediately stored in 4°C Krebs buffer (KB), transferred to the laboratory and used within 1 hour post mortem. The underlying tissue was removed with a scalpel blade and carefully trimmed with surgical scissors to achieve uniform thickness. During preparation the tissue was rinsed with 4°C KB every two minutes to prevent dehydration of the tissue. Prior to every experiment, MTT-tests were carried out to assure the viability of the tissue. As negative control, samples were boiled in water for one hour to deactivate the tissue (zero value). The measured values were calculated as absorbance units per mg tissue (i.e., tetrazolinium reductase index (TR Index)). Additionally, the integrity of the tissue was checked. The integrity test of the membrane was carried out using methylene blue/ PBS (1mg/ml) and methylene blue/ EDTA/ PBS (1mg/0.5mM/ml).The oral barrier studies were performed with static Franz diffusion cells (PermeGear, USA, 11.28 mm jacketed cell with a flat ground (ground o-ring) joint and clear glass with an 8 ml receptor volume). Each cell consisted of a donor and of a receiver compartment. The receiver compartment was surrounded by a water jacket to assure a physiological temperature of 37 ± 0.5 °C throughout the experiment. The receiver compartment was filled with 7.8 ml PBS buffer and heated to 37 °C before use. A magnetic stirrer was used with an agitation of 300 rpm to assure equal distribution. Between the compartments the excised viable and integral sheet of mucosa was inserted and fixed with retainer clips in such a way that the epithelium faces the donor and the connective tissue region faces the receiver compartment. After an equilibration time of 30 min, the buffer in the donor compartment was replaced by TiO2 particles dispersed in PBS in a concentration of 100 µg/ml. After 4 h test duration, the mucosa was washed 3 times with PBS, fixed and embedded. Observation of the tissue samples was carried out by transmission electron microscopy and the particles were verified by element-analyses. • Analytics (analytical verification) The penetration behavior of NM100 particles was evaluated by Transmission Electron Microscopy (TEM). The tissue was fixed with 0.1M sodium phosphate buffered 2.5% glutaraldehyde overnight at 4°C and post-fixed in 1.0% osmium tetroxide. Dehydration was carried out through a graded series of ethanol to 100%. Subsequently, the tissue was transferred into propylene oxide and embedded into epoxy resin. Thin tissue sections were cut with a diamond knife and placed onto 300 mesh copper grids. The grids were not stained with heavy metals to prevent staining precipitates. Transmission Electron Microscopy images were obtained using a TEM model Tecnai equipped with an energy filter.

Performance assessment of the method

Test materials

Details on test material

in media: Zeta-Potential: -24.0 mV agglomeration size: mean diameter 1600nm (PdI 0.104) RB adsorption constant: 0.026 ml/mg

Sample preparation/conditioning protocol

Particles dispersed in PBS and ultra-sonicated for 12h: mean diameter: 705.6 nm (PCS). Particle dispersion with a concentration of 0.4 mg/ml was prepared to determine the average particle size and the zeta potential. The particles were suspended in different physiological phosphate buffered saline. Ultra-sonication was carried out for 12 h to ensure a high particle distribution. The hydrodynamic size and zeta potential of the particles were measured by photon correlation spectroscopy (Malvern Zetasizer, Malvern Instruments) at a detection angle of 173°. The surface hydrophobicity was determined via the Rose Bengal (RB) adsorption method. The adsorbed amount of the hydrophobic dye Rose Bengal (Sigma Aldrich, Vienna, Austria) onto the particle surface was measured (Müller et al.). The particles dispersed in PBS were incubated at different RB concentrations (10-50 µg/ml) for 3 hours at room temperature. After centrifugation (3 hours at 14,000 rpm), the free amount of RB in the supernatant was measured spectro-photometrically at 544 nm (FLUOstar Optima, BMG Labortechnik). The maximal amount bound was determined using a Scatchard Plot. Thereby, the binding constant was calculated according as: r/a=KN-Kr where N is the maximum amount bound (µg/mg), r/a is the adsorbed amount of RB (µg/mg) per equilibrium concentration of RB (µg/ml) and K is the binding constant (ml/µg).

Results and discussions

Remarks on results including tables and figures

The results of the permeability studies demonstrated that TiO2 particles (NM105) were localized in the stratum superficiale and even in the deepest parts of the epithelium, the basal lamina. Thus, it could be demonstrated that NM105 particles can permeate the mucus layer and penetrate the buccal epithelium efficiently. In previous studies we demonstrated that the buccal uptake is a function of the surface charge, the size and hydrophilicity/hydrophobicity. It seems that in the case of NM 105 particles, the physicochemical properties such as particle size (35.8 nm), surface charge (-24.0 mV dispersed in PBS) and hydrophilicity/hydrophobicity (0.026 µg/ml dispersed in PBS) display a good prerequisite for an optimal uptake into the buccal mucosa. In previous studies it could be demonstrated that 200 nm positive and 200 nm neutral polystyrene nanoparticles were able to permeate the mucus layer readily and to penetrate the buccal epithelium to a high extent. 25 nm neutral particles also penetrated the mucosal tissue, however, with lower uptake efficiency and diffusion velocity than the larger ones. It seems that the mucus layer together with the buccal epithelium acts as a stronger barrier for smaller particles than for larger ones [1,2]. This phenomenon could be based on the special epithelial surface of the buccal mucosa, which is covered by ridge-like folds, so called microplicae. We hypothesized that these microplicae might enhance the effect of size-dependent particle uptake based on thermodynamic driving forces (and diffusion kinetics).

Overall remarks, attachments

Attached background material

figures_permeability NM105 P25_OECD.docx

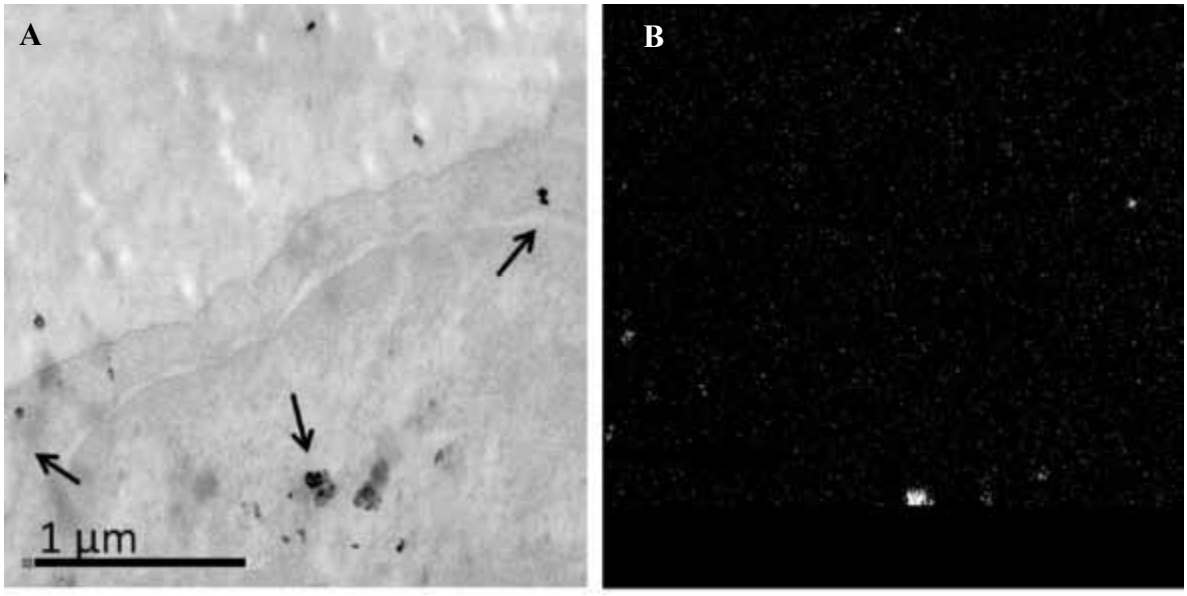


Figure 1 A) TEM image of NM 105 particles inside the superficial buccal epithelium. B) The existence of TiO_2 was verified by energy filtered TEM and the particles were verified as Ti and O_2.

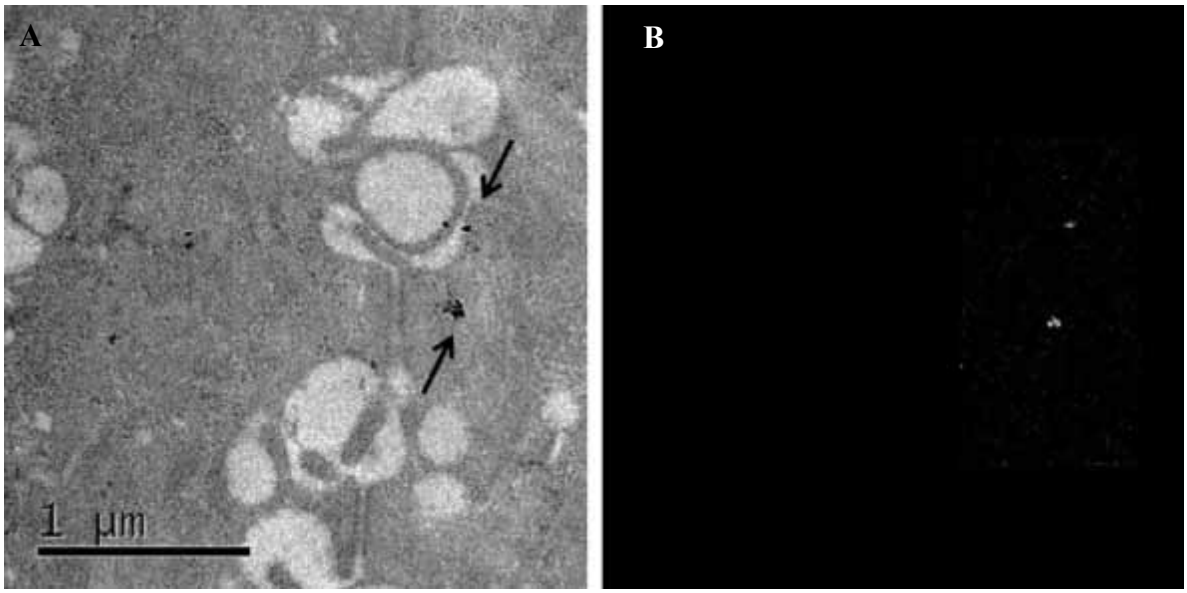


Figure 1 A) TEM image of NM 105 localized in the basal lamina. B) The existence of TiO_2 was verified by energy filtered TEM and the particles were verified as Ti and O_2.

Applicant's summary and conclusion

Conclusions

The results of the permeability studies demonstrated that TiO2 particles can permeate the mucus layer and penetrate deep parts of the buccal epithelium. Thus, it is essential to study possible translocation of NM105 into the systemic blood circulation and potential toxic effects in organs other than the port of entry..

Cross-reference to other study
[1] Roblegg E., Fröhlich E., Samberger C., Zaversky M., Teubl B., Zimmer A., Evaluation of a physiological in-vitro system to study the transport of nanoparticles through the buccal mucosa, Nanotoxicology (2012) 6, 399-413 [2] Teubl B., Meindl C., Eitzelmayer A., Zimmer A., Fröhlich E. and Roblegg E., In-vitro Permeability Studies of neutrally charged Polystyrene Particles through the Buccal Mucosa, small 2012, DOI: 10.1002/smll.201201789 [3] Teubl B., Leitinger G., Schneider M., Lehr C.M., Fröhlich E., Zimmer A., Roblegg E., The Buccal Mucosa as a Route for TiO2 Nanoparticle-Uptake, in preparation R.H. Müller, in Colloidal Carriers for Controlled Drug Delivery and Targeting, CRC Press Stuttgart. 1991 p. 99-109

Endpoint study record: cyto-toxicity by University of Graz

Administrative Data

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

Data source

Data access

other: performed and provided by University of Graz, Austria

Cross-reference to same study

Teubl B., Leitinger G., Fröhlich E., Schneider M., Tockner M., Zimmer A., Roblegg E., Buccal Mucosa as a Route for TiO2 Uptake, in preparation

Materials and methods

Principles of method if other than guideline

Method/ guideline followed: Roblegg E., Fröhlich E., Samberger C., Zaversky M., Teubl B., Zimmer A., Evaluation of a physiological in-vitro system to study the transport of nanoparticles through the buccal mucosa, Nanotoxicology (2012) 6, 399-413 Teubl B., Meindl C., Eitzelmayer A., Zimmer A., Fröhlich E. and Roblegg E., In-vitro Permeability Studies of neutral Polystyrene Particles through the Buccal Mucosa, small 2012, DOI: 10.1002/smll.201201789

Describe the scientific and technical basis of the test method

What biological/cellular model is the method based on?

buccal squamous epithelial TR 146 cells

What biological endpoints/responses does this method address?

cytotoxicity

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

Formazan bioreduction: In order to examine cell viability, a CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega) was used according to the instruction given by the manufacturer. 2 x 104 cells/200 µl medium were seeded in 96 well plates and cultured for 24 h. Subsequently, the medium was replaced by particles/serum-free medium dispersion in different concentrations and incubated for 4h

and 24h. 20 µl of a MTS/PMS solution per well was added and re-suspended. After an incubation time of 4 h, the absorbance was measured at 490 nm with a VIS-plate reader (FLUOstar Optima, BMG, Labortechnik). LDH release: To evaluate the lactate dehydrogenase (LDH) release, 2 x 104 cells/200 µl medium were seeded in a 96 well plate and incubated for 24 h. The medium was replaced by a particles/serum-free medium and incubated for 4h and 24h. LDH leakage was determined using a CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega) according to the manufacturer´s instruction. Control wells (100% LDH release) were treated with 2 µl of lysis solution. 25 µl of the supernatant were mixed with 25 µl of the CytoTox-ONE Reagent in a white microtiter plate. At the end of 10 min incubation time (at RT), reaction was stopped by adding 12.5 µl stop solution. The fluorescence was recorded by fluorometer (FLUOstar Optima, BMG, Labortechnik) at 560 nm excitation wavelength and 590 nm emission wavelength. Number of replica: n=6 Frequency of Dosing: - Positive and negative control groups and treatment: untreated cell as negative control Solvent: serum-free medium Description of follow up repeat study: same conditions Criteria for evaluating results: negative control, cell viability, seeding cell density (calculated from the gowth curve/prolifertation curve)

Describe the Standard Operating Procedure (SOP)

SOP description as a template

Sample administration: Particles dispersed in serum-free medium (n=6) •Exposure route: oral/buccal •Exposure duration: 4 and 24 h. •Concentration tested 1, 5, 10, 20, 50, 80, 100, 150, 200 µg/ml, (n=6)

Performance assessment of the method

Test materials

Sample preparation/conditioning protocol

Particles dispersed in serum-free medium

Results and discussions

Remarks on results including tables and figures

The cytotoxic effects of NM105 were assessed by a MTS assay after 4 and 24h. The data obtained from the particles showed that the mitochondrial activity/viability was not reduced (viability more than 90%), indicating no cytotoxic effects. At a particle concentration of 80 µg/ml and higher, NM 105 particles responded with a significant increased metabolic activity ($P<0.001$) after 4 h, indicating a stress situation of the cells. After 24 h incubation time, the metabolic activity returned to the level of untreated cells. The membrane integrity was assessed by LDH release. NM105 particles displayed no significant influence on the membrane integrity independent on the concentration within 24 h. The results of the MTS- and LDH-tests are listed in table 1 and 2 and illustrated in figure 5 and 6.

Overall remarks, attachments

Overall remarks

Generally, nanoparticles are effective disrupters of cell plasma membranes (Lerouei et al.). There are two common types of disruption, i) nanoscale hole formation and ii) membrane thinning effects. A disruption of the membrane correlates with the enzyme leakage, dye diffusion, cytotoxicity and in-vitro particle uptake. Therefore, LDH and MTS investigations were performed. The results indicated that no membrane disruption occurred. These data could be confirmed by the viability assays.

Attached background material
figures cytotoxicity_NM105 P25_OECD.docx

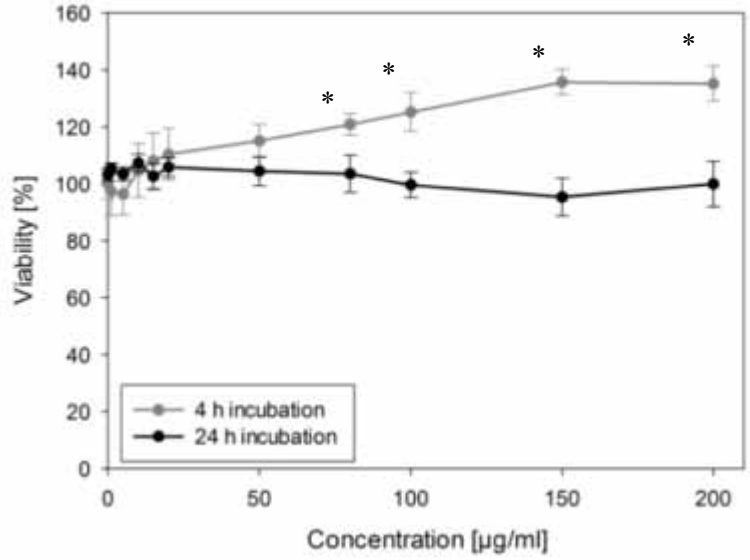


Figure 5 Viability assay (MTS-test) of TR 146 cells treated with NM105 particles (4 h and 24 h incubation time). * indicates a significant increased metabolic activity (P<0.001)

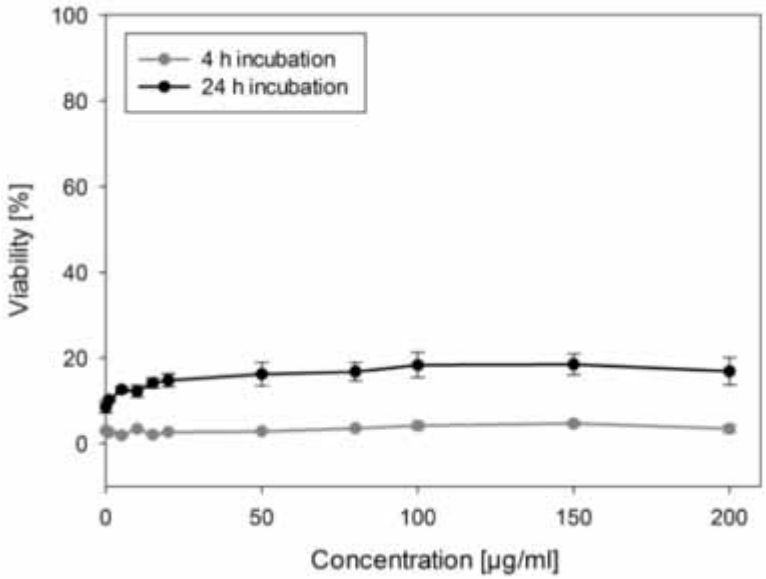


Figure 6 Membrane integrity assay (LDH-test) of TR 146 cells treated with NM105 particles (4h and 24 h incubation time).

Table 1 Results of the MTS-test of NM105

Concentration [µg/ml]	Viability [%] after 4 h	Standard deviation [%]	Viability [%] after 24 h	Standard deviation [%]
1	100,0000	5,2334	103,2227	2,1808
5	97,3224	8,4368	105,3713	1,9696
10	96,4002	7,2870	103,5320	2,3283
15	104,7238	9,4007	107,4143	3,0562
20	108,1333	9,7357	102,6630	4,6222
50	110,4521	9,0440	105,9056	3,3389
80	115,1195	5,7733	104,5293	5,0530
100	120,9651	3,7415	103,5524	6,5055
150	125,2685	6,8116	99,6660	4,4181
200	135,7679	4,4574	95,3933	6,5826

Table 2 Results of the LDH-test of NM105

Concentration [µg/ml]	LDH release [%] after 4 h	Standard deviation [%]	LDH release [%] after 24 h	Standard deviation [%]
1	3,0328	0,0827	8,4877	1,3435
5	2,6314	0,1234	10,2075	0,4625
10	1,9178	0,2086	12,6103	0,6593
15	3,4450	0,1069	12,2079	1,3963
20	2,0759	0,2630	14,1370	1,1460
50	2,7072	0,2191	14,7479	1,5141
80	2,8806	0,1978	16,1747	2,7235
100	3,5573	0,2792	16,7677	2,1467
150	4,1773	0,7566	18,3318	2,9237
200	4,6600	0,5349	18,4924	2,4142

Applicant's summary and conclusion

Conclusions

NM105 particles, tested in concentrations up to 200 µg/ml, do not affect the viability and the membrane integrity of human buccal epithelial cells under in-vitro conditions, but they may induce intracellular ROS production. (manuscript in preparation)

Cross-reference to other study

Leroueil P.R., Hong S., Mecke A., Baker J.R., Orr B.G., Banaszak Holl M.M., Acc. Chem. Res. 2007, 40, 335-342 Roblegg E., Fröhlich E., Samberger C., Zaversky M., Teubl B., Zimmer A., Evaluation of a physiological in-vitro system to study the transport of nanoparticles through the buccal mucosa, Nanotoxicology (2012) 6, 399-413 Teubl B., Meindl C., Eitzelmayer A., Zimmer A., Fröhlich E. and Roblegg E., In-vitro Permeability Studies of neutral Polystyrene Particles through the Buccal Mucosa, small 2012, DOI: 10.1002/smll.201201789

8. ANALYTICAL METHODS

9. RESIDUES IN FOOD AND FEEDINGSTUFFS

10. EFFECTIVENESS AGAINST TARGET ORGANISMS

11. GUIDANCE ON SAFE USE