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English - Or. English

ENVIRONMENT DIRECTORATE
CHEMICALS COMMITTEE

ENV/CHEM/NANO(2009)4/ADD6
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Working Party on Manufactured Nanomaterials

DRAFT DOSSIER DEVELOPMENT PLAN: TITANIUM DIOXIDE

5th Meeting of The Working Party on Manufactured Nanomaterials taking place at OECD Headquarters in Paris, France on 4-6 March 2009, starting at 10h00 on the first day.

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This is an addendum to the Report on the Sponsorship Programme for the Testing of Manufactured Nanomaterials [ENV/CHEM/NANO(2009)4].

This document is a Draft Dossier Development Plan (DDP) for titanium dioxide. The drafting of this document is being lead by Germany and France.

ACTION REQUIRED: ***The WPMN is invited to take note.***

DDP OF TITANIUM DIOXIDE

I. Introduction

The background of the Titanium Dioxide Testing Programme will be provided after the review of tests described in chapter V has been completed.

II. Identification of Participants

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Coordination and responsible sponsor of each test

The coordination of each test will be according to the agreement among participants and researchers (see researcher contact information below). The coordination of each endpoint will be according to the agreement among participants.

Responsible Sponsors for final Report to WPMN

Lead Sponsors (Germany and France) are responsible for making the final report to the WPMN on their own part. The decision of which part Lead Sponsors will be responsible for will be made after evaluation of the projects (see Chapter V).

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III. Communication Strategy

Teleconferences amongst sponsors will be held periodically. For information exchange of the planned projects and projects in progress a spreadsheet was provided by the lead sponsor (Annex 1). The communication between sponsors, lead sponsors and contributors will be done by e-mail. It was also agreed that the OECD password protected website will be used for communication between the Lead-Sponsors, Co-Sponsors and Contributors.

Teleconferences/Meetings:

Two teleconferences were held before the Korea meeting. Details see below and in the agendas on the OECD password protected website.

1. Teleconference, 2008, September, 25th

   Main agenda items:

   - Process of communication, collaboration, and organization
   - Timeframe for the next steps
   - Initial discussion of which endpoints sponsors/co-sponsors wish to test

2. Teleconference, 2008, November, 12th

   Main agenda items:

   - Discussion of the DDP Draft
   - Korea meeting

3. Teleconference, 2009, January 14th

   Main agenda items:

   - Discussion of the DDP Draft, rev version 2
   - Discussion of the alternatives Material Selection

IV. Material Selection

Most of the tests up to now have been carried out with Degussa/Evonik P 25. Thus, Sponsors, Co-Sponsors and Contributors (Korea and Evonik missing) agreed (Teleconference, Sept, 25th) the nanomaterial which should be tested is **Degussa/Evonik P 25**. This material is used worldwide because of its photocatalytic effects. P 25 should be used as a standard/reference material for all endpoints, if possible. It was also agreed that experiments should be conducted with other Nano- TiO_2-samples in order to investigate the dependence of test results on crystal form, size and coating. **For the Sponsorship Programme P25 will be distributed by Evonik**.

As agreed in the teleconference September 25th for comparing and a better understanding of the test results the following information should be included for each test:

- The preparation of the material for testing (e.g. to notice possible effects of ultrasonic treatment)

- The batch number, if available (the properties of different batches may slightly vary within the given product specification)

As agreed by participants during the workshop in Korea and in the teleconferenc January 14th, the DDP should include P25 but also alternative in order to. cover all the uses of TiO_2 NMs.Not all but the most relevant end-point will be tested to set a sort of correlation between physico-chemistry propeties reactivity and risk for environnemental and health assesment. After a french solicitation, the Titanium

Dioxide Manufacturers Association[1] has decided to join the sponsorship programme. The assciation, after consultation and agrement of the consortium, send to France a list of product for wich they took the engagement of providing data and samples. These products are commercially available and covering all applications of titanium oxide nanaomanufatcured (see material 20).

In the teleconference January 14th, it was reached an agreement that previously to the selection of alternative materials, some preliminary tests focusing on characterization, and especially physico-chemical properties (particle size, specific surface area,…). In that purpose, all commercially available materials, inlcuding P25, should be tested with same conditions and methods by independent laborotaries. This previous horizontal work wil have two main benefits :

- To ensure that material sold an used in test or experiments are similar : sometimes there is difference between the product oredered and received.
- To set up a solid data base which should offer the opportunities to compare different tests in toxycology, ecotoxicology, which is actualy quite difficult.

Other materials used in tests which are or will be in progress:

Material 1
amorphous titanium dioxide; count median diameter **22nm**, geometric standard deviation 1.7;

Material 2
T-lite SF, rutile, primary particle: length: 50; **width: 10 nm, coated** with aluminum hydroxide (and) dimethicone/methicone copolymer

Material 3
T-lite SF-S, rutile, primary particle: length: 50; **width: 10 nm**, **coated** with hydrated silica (and) dimethicone/methicone copolymer (and) aluminum hydroxide

Material 4
T-lite max, rutile, primary particle: length: 50; **width: 10 nm, coated** with dimethoxydiphenylsilane/triethoxy-caprylylsilane crosspolymer (and) hydrated silica (and) aluminum hydroxide

Material 5
Hombicat UV 100, (untreated Anatase), **100 nm**

Material 6
P 805: **Coating:** Triethoxyoctylsilyl groups or trimethyloxy-octylsilane groups

Material 7
5 nm Stock number: 44689; Lot number: I09Q40;
Producer: Alfa Aesar GmbH & Co KG, Karlsruhe, Germany

[1] TDMA membership :

Full members: Cinkarna Celje.d.d,. (SI) Evonik, (DE) Huntsman Tioxide Europe (GB, ES, FR, IT), Kemira Pigments Oy (FI), Kronos Worldwide Inc. (BE, DE, NO), Millennium Chemicals (GB, FR), Precheza AS (CZ), Sachtleben Chemie GmbH (DE), Tronox Pigments International GmbH (DE, NL), Zaklady Chemiczne POLICE S.A. (PL), Associate members: DuPont de Nemours (USA), Ishihara Sangyo Kaisha, Ltd. (Japan), Tayca (Japan)

Material 8
NanoAmor 5nm Anatase

Material 9
NanoAmor 10 nm Anatase

Material 10
Rutile (30 nm) -> -> Manufacturer? (used by Canada)
***Material* 10.a** NanoAmor, rutile (30-40 nm) used by US EPA)

Material 11
Anatase (44 μm)

Material 12
DuPont™ Light Stabilizer 210 and 220
(DLS 210, DLS 220), 135 nm (wt basis), polyhedral, predominantly rutile, surface treated.

Material 13
Millenium

Material 14
Kemira RDI-S (220 nm rutile TiO2) coated with Al2O3 and organic compound

Material 15
W2730X (<100 nm primary particles **suspended in water**)

Material 16
UV Titan L 181 (appr. **17 nm**) TiO2 **coated** with Al, Si, Zr and polyalcohol

***Material 17* Materials used by US EPA:**
Material P 25
Material 17-2. 10 nm, anatase, Alfa Aesar, Catalog #44690
Material 17-3. 32 nm, anatase, Alfta Aesar, Catalog #39953
Material 17-4. 30 – 40 nm, rutile, Nano Amor,
Material 17-5. 200 nm, anatase, Acros, Catalog #21358
Material 17-6. 250 nm, rutile, MK Nano, Catalog #MKN-TiO2-R250

For more information about the various types of TiO_2 used by the US EPA see Annex 3.

Material 18
T805. rutile/anatase; silicone dioxide <2.5%; 21 nm. Degussa. (This preparation has been allotted the CAS number 100209-12-9)

Material 19
uf-TiO2 1, rutile, 136.0 ±47.6 nm *(Hydrodynamic diameter), 98%* TiO2*, O.2% alumina and* uf-TiO2 2, rutile, 149.4 ±74.7 nm *(Hydrodynamic diameter),* 88.0% TiO2 5.0% alumina 7.0 amorphous silica

Material 20 Materials from TDMA (in addition of P25)
Material 20-1. **Hombitan R 320**; untreated rutile, 5-20m2/g, Sachtleben (Pigment for control)

Material 20-2. **UV-TITAN M212 and M262**, surface treated rutile, surface area 50-150m2/g, Sachtleben
Material 20-3. **Tiona AT-1**, untreated anatase, surface area 5-15m2/g**,** Cristal Global, (Pigment for control)
Material 20-4**. KRONOS 1001**, untreated anatase, surface area 5-15m2/g, KRONOS
Material 20-5. **PC-105**, untreated anatase, surface area 50-200m2/g, Cristal Global
Material 20-6. **KRONOS vlp 7000 and uvlp 7500**, untreated anatase, surface area 200-350m2/g, KRONOS
Material 20-7. **Tronox 8600**, untreated anatase, surface area 200-350m2/g, Tronox

Material 21
Several other materials used by Fraunhofer IME, Germany: differing in properties. The applied substances will be specified

V. Endpoints for testing Titanium Dioxide

A spreadsheet for communication of the planned contributions was distributed to the participants by Germany. The spreadsheets give an overview about the existing projects with respect to Titanium Dioxide and the projects in progress. The following information is based on the completed spreadsheets.

The data below list the projects (finished, planned and in progress) for the different endpoints. These projects were evaluated by sponsors and co-sponsors before the 5th WPMN meeting. Gaps than were identified, responsibilities for each Endpoint were fixed then. At the 2nd teleconference was agreed to prepare a reference paper for identifying which endpoints are being addressed by whom. This would allow TiO2 participants as well as others to see where there are gaps. This paper is added to the DDP as Annex 2.

Nanomaterial information/identification

NOTE: All TiO2 particles have undergone independent physical, chemical, and biological analysis for purity, primary size, aggregate size range, surface area, crystalline structure, and endotoxin content.

1. Nanomaterial name:

   Titanium Dioxide (TiO2)

2. CAS Number:

   13463-67-7

3. Structural formula/molecular structure:

   Empirical Formula: **TiO_2**

4. Composition of nanomaterial being tested:

P25:
1. Evonik:
85% anatase, 15% rutile
2. US EPA:
86% anatase, 14% rutile

Material 1
amorphous titanium dioxide;

Material 2
rutile, primary particle coated

Material 3
rutile, coated

Material 4
rutile, coated

Material 5

Material 6
Titanium oxide coated

Material 7
Titanium oxide

Material 8
Anatase

Material 9
Anatase

Material 10
Rutile

***Material* 10a**
rutile

Material 11
Anatase

Material 12
rutile, coated.

Material 13
anatase

Material 14
rutile coated

Material 15
No information

Material 16
TiO2 coated

Material 17

Material 17-2.anatase,
Material 17-3. anatase
Material 17-4. rutile
Material 17-5. anatase
Material 17-6. rutile,

Material 18
rutile/anatase; coated

Material 19
uf-TiO_2 1, rutile, coated
uf-TiO_2 2, rutile, coated

Material 20
Material 20-1. rutile,
Material 20-2. rutile
Material 20-3. anatase,
Material 20-4. anatase
Material 20-5. anatase
Material 20-6. anatase
Material 20-7. anatase

Material 21
Information will be provided later

5. Basic morphology

P25:
1. Evonik:
count median diameter **22nm**, geometric standard deviation 1.7; strongly aggregated and agglomerated
2. US EPA:
Primary particle Size: **21 nm**; size range: 14,2-64,6 nm,
Material 1
count median diameter **22nm**, geometric standard deviation 1.7;

Material 2
primary particle: length: 50; width: 10 nm**,**

Material 3
primary particle: length: 50; width: 10 nm,

Material 4
primary particle: length: 50; width: 10 nm**,**

Material 5
primary particle 100 nm

Material 6
No information

Material 7
primary particle 5 nm

Material 8
primary particle 5nm

Material 9
primary particle 10 nm

Material 10
primary particle 30 nm
***Material* 10.a** primary particle 30-40 nm

Material 11
primary particle? 44 μm

Material 12
135 nm (wt basis), polyhedral
Additional characterization can be found in the following publication: *Warheit DB, Webb TR, Reed KL, Frerichs S, and Sayes CM. Pulmonary Toxicity Study in Rats with Three Forms of ultrafine-TiO2 Particles: Differential Responses related to Surface Properties. Toxicology 230:90-104, 2007; 2006 Nov 10;*

Material 13
No information

Material 14
primary particle? 220 nm

Material 15
primary particle <100 nm

Material 16
primary particle 17 nm

***Material 17* Materials used by US EPA:**
Material P 25
Material 17-2. primary particle 10 nm
Material 17-3. primary particle 32 nm,
Material 17-4. primary particle 30 – 40 nm
Material 17-5. primary particle? 200 nm, rutile, MK Nano, Catalog #MKN-TiO2-R250

Material 18
primary particle 21 nm

Material 19
uf-TiO2 1, 136.0 ±47.6 nm *(Hydrodynamic diameter*
uf-TiO2 2, rutile, 149.4 ±74.7 nm *(Hydrodynamic diameter),*

Material 20
No information

Material 21
Information will be provided later

6. Description of surface chemistry (e.g. coating or modification)

material 4
coated with dimethoxydiphenylsilane/triethoxy-caprylylsilane crosspolymer (and) hydrated silica (and) aluminum hydroxide (provided by BASF SE)

material 6
Triethoxyoctylsilyl groups or trimethyloxy-octylsilane groups

Material 12
with aluminum hydroxide, amorphous silica, and a polyol, silane or siloxane.

Material 2
coated with aluminum hydroxide (and) dimethicone/methicone copolymer

Material 3
coated with hydrated silica (and) dimethicone/methicone copolymer (and) aluminum hydroxide

Material 4
coated with dimethoxydiphenylsilane/triethoxy-caprylylsilane crosspolymer (and) hydrated silica (and) aluminum hydroxide

Material 6
coated with Triethoxyoctylsilyl groups or trimethyloxy-octylsilane groups

Material 11
Anatase (44 μm)

Material 12
Treated with aluminum hydroxide, amorphous silica, and a polyol, silane or siloxane.

Material 14
Coated with Al2O3 and organic compound

Material 16
Coated with Al, Si, Zr and polyalcohol

Material 17

Material 19
uf-TiO_2 1, coated with O.2**%** alumina
uf-TiO_2 2coated with 5.0% alumina 7.0 amorphous silica

Material 20

Material 20-2. surface treated but no information on it

Material 21
Information will be provided later

7. Major commercial uses

P 25:
Photocatalytic effects, but also in cosmetics without in this case the agreement of the manufactrurer Evonik

Material 12
Photocatalytic effects

Material 20
Material 20-1. sunscreen and cosmetic and photocatalytic effects (photocatalyst and DeNOx)
Material 20-2. sunscreen and cosmetic
Material 20-3. photocatalytic effects (photocatalyst and DeNOx)
Material 20-4. photocatalytic effects (photocatalyst and DeNOx)
Material 20-5. photocatalytic effects (photocatalyst and DeNOx)
Material 20-6. photocatalytic effects (photocatalyst and DeNOx)
Material 20-7. photocatalytic effects (photocatalyst and DeNOx)

8. Known catalytic activity

P 25:
photocatalytic activity

Material 12
Material has been formulated to suppress catalytically active through crystal formation methods and surface treatment. Additive to polymers for UV blocking

Material 20
Material 20-1. UV blocking and photocatalytic activity (photocatalyst and DeNOx)
Material 20-2. UV blocking
Material 20-3. photocatalytic activity (photocatalyst and DeNOx)
Material 20-4. photocatalytic activity (photocatalyst and DeNOx)
Material 20-5. photocatalytic activity (photocatalyst and DeNOx)
Material 20-6. photocatalytic activity (photocatalyst and DeNOx)
Material 20-7. photocatalytic activity (photocatalyst and DeNOx)

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

P 25:
gas phase hydrolysis

Material 12
Confidential Business Information

For other materials, information will provided later

Physical-chemical properties and material characterization

NOTE :

US EPA: Physical and chemical analyses of 7 different titanium dioxide particles performed to assess:

- purity - qualitative and quantitative elemental determination;
- specific surface area;
- primary and aggregate size determination;
- crystal structure;
- elemental and organic carbon content;
- particle shape and morphology.
- endotoxin content

The following suggested list of specifications could be used to evaluate responses from vendors submitting proposals for the requested service and as it relates to meeting the scope of work. The vendor must:

- qualitative elemental analysis as determined by proton induced X-ray emission (PIXE);
- quantitative elemental analysis as determined by ion couple plasma mass spectroscopy (ICPMS);
- specific surface area as determined by Brunauer, Emmett, Teller test (BET);
- primary and aggregate size determination as determined by transmission electron microscopy (TEM);
- crystal structure as determined by X-ray diffraction (XRD);
- elemental and organic carbon content as detemined by the thermal optical analysis for organic and elemental carbon assay based on NIOSH 5040 method or equivalent method of analysis.
- particle shape and morphology as determined from transmission electron microscopy (TEM) and Brunaeuer Emmett Teller test (BET);
- endotoxin presence or absence as determined by an endotoxin specific turbidmetric assay following pre-tests to ensure that TiO2 do not by themselves alter or influence the turbimetric test.

1. Agglomeration/aggregation

P25:
agglomeration was usually detected before and after testing (more information will given later in the chapters of test description);

Material 1:
chain aggregates/agglomerates with primary particles of 2-4 nm

Material 12
Agglomerated - Material is normally agglomerated in powder form and is dispersed in the polymer melt as it is being incorporated into the product.

Materials 20
All are aggregated/agglomerated

2. Water solubility

P 25:
Insoluble

Material 12
Insoluble

Other Endpoints:

Dispersion stabilility in Water

(Fraunhofer IKTS and other)

3. Crystalline phase

P 25:
no cristalinity for TiO2 but only amorphous structures (described for other material 1)
Crystal form: 86% anatase, 14% rutile (Lot No 4165012298)

Material 12
Crystalline Rutile with a minor amount of anatase

Materials 20
informations will be provided later

4. Dustiness

P25:
Data may be available from NanoCare

Materials 20
informations will be provided later

5. Average crystallite size

P25.
21 nm

Material 12
Particle size D50 is ~130-140 nm on a weight basis via dynamic light scattering after dispersion in water with a surfactant and sonication. Crystallite size reported by X-ray Diffraction is most often smaller than actual particle size.

Materials 20
informations will be provided later

6. Representative TEM picture(s)

P25:
TEM pictures for various studies are available
Material 12
A micrograph can be provided

Materials 20
informations will be provided later

7. Particle size distribution – dry and in relevant media

P25:
available for TiO2 and other materials. The size will be obtained by measuring with TEM and/or REM

Material 12
Particle size D50 is ~130-140 nm on a weight basis via dynamic light scattering after dispersion in water with a surfactant and sonication. The geometric standard deviation is ~1.3 to 1.4.

Materials 20
informations will be provided later

8. Specific surface area

P25:
measured by. BET

M
aterial 12
3—60 m^2/g via Nitrogen Adsorption (BET)

Material 20
Material 20-1. 5-20 m^2/g
Material 20-2. 50-150 m^2/g
Material 20-3. 5-15 m^2/g
Material 20-4. 5-15m^2/g
Material 20-5. 50-200m^2/g
Material 20-6. 200-350m^2/g
Material 20-7. 200-350m^2/g
Informations on the method used will be provided later
Material 21
Information will be provided later

9. Zeta potential (surface charge)

P25:
Data available

Material 12
Zeta Potential at 7.0 pH = 17, Isoelectric Point = 7.7

Materials 20
information will be provided later

10. Surface chemistry (where appropriate)

Materials 20
s

Material 21
Information will be provided later

11. Photocatalytic activity

Dr. Paul Howard, US FDA, has agreed to perform photocatalytic measurements of TiO2 particles if they are supplied to him.

Material 12
Low/photopassivated

Materials 20
information will be provided later

Material 21
Information will be provided later

12. Pour density

Material 12
Loose bulk density is 400-480 kg/m3 (25-30 lb/ft3)

Materials 20
information will be provided later

13. Porosity

P25:
Very low due to sintering during gas phase production at high temperatures

Material 12
Average pore diameter = 85-107 Angstroms, Pore volume = 0.11 cc/g

Materials 20
information will be provided later

14. Octanol-water partition coefficient, where relevant

This item is not relevant to P25 particles, because TiO2 is insoluble in Water.

Material 12

This item is not relevant to this material, because TiO2 is insoluble in water

15. Redox potential

This item is not relevant to P 25.

16. Radical formation potential (including surface properties)

Material 12
Low/photopassivated

Materials 20
information will be provided later

Other relevant information (where available)

Environmental fate

Biodegradation

There is no biodegradation of Titanium Dioxide, but information about substances which are produced by photocatalytic effects should be given.

Germany (planned project)

Sedimentation and surface water transport

P 25:

Country / Organisation: Austria
Responsible: Vienna University
Status: completed
Year of publication or finishing:
Method:
Characterization in a laboratory flume with and without biofilm as sediment coating; biofilm microorganisms damage: amount of dead cells in biofilm; radical formation; range of transport; surface characterization; ROS detector, carboxy-H2DCFDA, life/dead-kits at the biofilm and the batch, clean-up by dispersion/centrifugation, dispersion with ultrasonication and separation of small particle fraction (<<1µm) by settling (48h), concentration: 5 mg/L, static light scattering, dynamic light scattering, zeta potential, ICP-MS screening, ICP-MS Ti analysis, Raman microscopy, transmission x-ray microscopy
Sample preparation method:
Results:
Comments

Material 5:

Country / Organisation: Austria
Responsible: Vienna University
Status: completed
Year of publication or finishing:
Method:
Same study like 1.), different material.
Characterization in a laboratory flume with and without biofilm as sediment coating; biofilm microorganisms damage: amount of dead cells in biofilm; radical formation; range of transport; surface characterization; ROS detector, carboxy-H2DCFDA, life/dead-kits at the biofilm and the batch, clean-up by dispersion/centrifugation, dispersion with ultrasonication and separation of small particle fraction (<<1µm) by settling (48h), concentration: 5 mg/L, static light scattering, dynamic light scattering, zeta potential, ICP-MS screening, ICP-MS Ti analysis, Raman microscopy, transmission x-ray microscopy
Sample preparation method:
Results:
Comments

Aggregation in surface water

P 25:

Country / Organisation: Austria
Responsible: Vienna University
Status: completed
Year of publication or finishing:
Method:
same study like 1.) different material
Find key-parameter for aggregation in (surface) water (e.g. pH, humic substances, salinity, different major ions, DOC) determine concentration of stable fraction, particle size, zeta potential as a function of water chemistry (completed)
Method: ICP-MS Ti analysis from "as particle" and digested samples; agglomeration/aggregation during the test: Measuring for media containing calcium, sodium, sulphate no for media containing pyrophosphate, low for media containing sodium at low pH, low for media containing humic acid addition
Sample preparation method:
Results:
Comments

Material 5:

Country / Organisation: Austria
Responsible: Vienna University
Status: completed
Year of publication or finishing:
Method:
same study like 1.) different material

Find key-parameter for aggregation in (surface) water (e.g. pH, humic substances, salinity, different major ions, DOC) determine concentration of stable fraction, particle size, zeta potential as a function of water chemistry (completed)
Method: ICP-MS Ti analysis from "as particle" and digested samples; agglomeration/aggregation during the test: Measuring for media containing calcium, sodium, sulphate no for media containing pyrophosphate, low for media containing sodium at low pH, low for media containing humic acid addition
Sample preparation method:
Results:
Comments

Material 8:

Country / Organisation: Canada
Responsible: MC Gill University
Status: planned
Year of publication or finishing:
Method:
Aggregation and dissolution in various aqueous media, role of NOM, PO4, Ca, pH, ionic strength Sample Preparation Method: Under development, focusing on stirring first, avoiding solvents or dispersants, allowing samples to stabilize O/N; concentration of Test substance: 1 -10 mg/L
Sample preparation method:
Results:
Comments

Transport in sands and soils

Material 8

Country / Organisation: Canada
Responsible: MC Gill University
Status: in progress
Year of publication or finishing:
Method:
Sample preparation method:
Results:
Adaptation of Test Method: Currently developing methods for dispersing material in water that are consistent and repeatable - also adding organic matter as treatment. Sample Preparation Method: Under development, focusing on stirring first, avoiding solvents or dispersants, allowing samples to stabilize O/N; concentration of Test substance: 1 -10 mg/L
Comments

Adsorption/Desorption

P 25:

Country / Organisation: Austria
Responsible: Vienna University
Status: planned
Year of publication or finishing:
Method:
Sample preparation method:
Results:
Comments

Hydrolysis

P 25:

Country / Organisation: Austria
Responsible: Vienna University
Status: planned
Year of publication or finishing:
Method:
Sample preparation method:
Results:
Comments

Emission into environment

Country-Organisation : published Data
Responsible:
Status: published
Year of publication or finishing: 2008
Method:
Facade runoff from a building painted in May 2006 (referred to as aged facade), was collected with an aluminum gutter (1.0 m length) mounted at the bottom of the facades' drain rail. The runoff drained into a plastic bottle. The spacing between the facade and the aluminum gutter was kept as minimal as possible at a distance of approximately 2–3 mmin order to avoid dilution with additional rain water. After each runoff event, samples were directly taken to the laboratory and processed within a few hours. In addition to that building, runoff from a new facade (an experimental building area, 1.3 m2) painted with the same product was studied.
The experimental setup including the collection and preparation procedures is described in the article.All samples for TEM and ICPMS analysis were derived from the same, well-mixed batch water sample collected over the period of one complete rain event.
Sample preparation method:
The focus of this study was on synthetic NPs of TiO2 with a size of up to a few 100 nm. Optimizing

the preparation procedure to selectively deposit these particles on TEM grids and to remove larger particles which could negatively interfere (large particles deposited on the smaller ones) with the particles of interest. The setup consisted of a two stage centrifugation procedure (Megafuge, Heraeus, equipped with a swinging bucket rotor). During the first step, larger particles are removed. Just after the centrifugation the uppermost supernatant (2 cm, 40 mL) of the centrifuge tubes is removed using a peristaltic pump. This supernatant containing TiO2 particles <300 nm, was centrifuged again for 2 h at 2700 _ g which resulted in a total deposition of TiO2 particles larger than about 20 nm. More details : see publication

Test organism:

Results:

Synthetic TiO2 particles within a size range of a few tens to a few hundreds of nm in diameter were successfully detected and identified in the environment using a combination of analytical electron microscopy (TEM-EDX) and bulk chemical (ICP-MS) methods. Based on this information, we were able to give a rough estimate of the number of synthetic TiO2 NPs in runoff samples. This is the first step towards an assessment of released NPs in environmental samples. An evaluation of the number or mass flux of synthetic NPs in urban runoff further requires data on dynamics of individual runoff events as well as on other urban transport pathways to the aquatic environment.

Based on a modeling study, Mueller and Nowack, 2008 concluded that the nano-TiO2 particles in water may pose a risk to aquatic life. In this study we show for the first time that TiO2 particles are released in significant amounts to the aquatic environment.The rather fast surface runoff under heavy rainfall conditions may transport NPs without significant retention mechanisms, which inevitably leads to a discharge of synthetic NPs into surface waters.

Comments :

Environmental Pollution 156 (2008) 233–239, Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment

R. Kaegi, A. Ulrich, B. Sinnet, R. Vonbank, A. Wichser, S. Zuleeg, H. Simmler, S. Brunner, H. Vonmont, M. Burkhardt, M. Boller

Others end-points

P 25:

Country-Organisation : France/CEA

Responsible: Francois Tardif

Status:, in progress

Year of publication or finishing:

Method: Release-ability of nano TiO2 from matrix of future nanoproducts, creams, etc.

Commercial TiO2 (Degusa) and home made synthetised TiO2 by hydrothermal and supercritical process. Sizes ranging from 20 to 500 nm

Sample preparation method:

Test organism:

Results:

Comments

P25, and others

Country-Organisation : France/ CEA Saclay
Responsible: Marie Carriere, Nathalie Herlin
Status: in progress
Year of publication or finishing:
Method: Surface modification (XAS)
TiO2 purchased from sigma, degussa and synthesized by laser pyrolysis in DSM/IRAMIS/SPAM laboratory (CEA Saclay)
Sample preparation method: cell culture media - hoagland medium
Test organism:
Results:
Comments :

Environmental toxicology

1. Effects on pelagic species (short term/long term)

This endpoint will also be addressed by Germany, University of Bremen

2. Toxicity to Algae

P 25:

growth inhibition test

Country / Organisation: Germany
Responsible: Fraunhofer IME, Kerstin Hund-Rinke:
Status: published
Year of publication or finishing:
Method:
OECD test medium, no standard test
Sample preparation method:
TiO2 was dispersed in algae test medium (prepared according to the guideline) in a 50-fold concentration. Dispersion was achieved by ultrasonic dispersion. 1 mL of the dispersion was diluted (1:50) with algae adjusted to a concentration of 10,000 cells/ml in test medium according to the guideline.
Test organism: Desmodesmus subspicata
Results:
Comments

Country / Organisation: Spain
Responsible: INIA
Status: planned
Year of publication or finishing:
Method:
OECD 201 Test Guideline, Reference material: The use of K2TiO(C2O4)2·2H2O is under discussion,
Sample preparation method:
Test organism: Chlorella vulgaris, Selenastrum capricornutum
Results:
Comments

Material 5

Country/ Organisation: Germany
Responsible: Fraunhofer IME, Kerstin Hund-Rinke: –
Status: published,
Year of publication or finishing:
Method:

OECD test medium (same study like P25, different material)
Sample preparation method: TiO2 was dispersed in algae test medium (prepared according to the guideline) in a 50-fold concentration. Dispersion was achieved by ultrasonic dispersion. 1 mL of the dispersion was diluted (1:50) with algae adjusted to a concentration of 10,000 cells/mL in test medium according to the guideline.
Test organism: Desmodesmus subspicata
Results:
Comments :

Material 8, 9, 10, 11

Country / Organisation: Canada
Responsible: BRI
Status: in progress / planned
Year of publication or finishing:
Method:
Growth inhibition and FacScan-type analyses: Agglomerate versus algae based on chlorophyll Method: 96 h- growth inhibtion using microplate method, Envir Canada (1992), concentration of test substance: from 0.02 to 100 mg/L. Measuring; Purity, Crystal composition, Bulk grain Size, Aspect, Calc SA, BET SA, Zeta mV,
Test organism: Pseudokirchneriella subcapitata
Results:
Comments

Material 12:

Country / Organisation: BIAC
Responsible: Du Pont
Status: published
Year of publication or finishing: 2007
Method: OECD 201 Static, Acute, 72-Hour Growth Inhibition Toxicity Screening Test to the Green Algae
Sample preparation method: The acute toxicity of a DuPont Light Stabilizer prototype (uf-C) titanium dioxide particles to the green algae, Pseudokirchneriella subcapitata, was determined in a 72-hour, static toxicity test according to OECD 201 testing guidelines (OECD).
The study was conducted with a synthetic algal-assay procedure (AAP) nutrient medium blank control and 5 concentrations of fine (pigmentary) or ultrafine TiO2 particles at a mean lighting intensity of 8938 lux (range of 8200 to 9500 lux), a mean temperature of and a dilution water control at a mean temperature of 23.8 oC (range of 23.7 to 23.8 oC) and a shaking speed of 100 rpm. Two replicates were used per blank control and test concentration each with an initial cell count (density) of 10,000 cells/mL. Based on visual observations the 100 mg/L test concentration solutions were very cloudy with suspended substance present at test start. The 10 mg/L test concentration solutions were slightly cloudy with suspended substance present at test start. The blank control and remaining test concentration solutions were clear and colorless with no visible precipitate at test start. All environmental parameters were within acceptable limits during the exposure.
Exposure of algae to nominal concentrations of 0.01, 0.1, 1, 10 and 100 mg/L fine (pigmentary) TiO2 particles resulted in -2, 3, 2, 31, and 97% inhibition, respectively, based on healthy cell count compared to the blank control at the end of 72 hours; percent inhibition of growth rate was 0, 1, 0, 8, and 66%, respectively. Exposure of algae to nominal concentrations of 0.01, 0.1, 1, 10, and 100

mg/L DLS TiO2 particles resulted in -19, -6, -11, 15, and 94% inhibition, respectively, based on healthy cell count compared to the blank control at the end of 72 hours; percent inhibition of growth rate was -3, -1, -2, 3, and 54%, respectively. Healthy cell counts increased in the blank controls by at least a factor of 16 in 72 hours, thereby satisfying the appropriate test acceptance criteria.
The algae 72-hour EC50 values (95% fiducial limits) based on inhibition of growth and healthy cell counts were 16 (12 to 22) mg/L for fine (pigmentary) TiO2 particles and 21 (16 to 26) mg/L for DLS TiO2 particles (uf-C). The 72-hour EC50 values (95% fiducial limits) for growth rate based on nominal concentrations and healthy cell counts were 61 (52 to 72) mg/L for fine-TiO2 particles and 87 (83 to 91) mg/L for DLS TiO2 particles.
Test organism: Green algae, Pseudokirchneriella subcapitata
Results:
Results demonstrated that fine (Pigmentary) and TiO2 particles exhibited medium concern under TSCA in a 72-hour, acute test (Smrchek et al., 1993).
Comments : Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

Material 21

Country / Organisation: Germany
Responsible: Kerstin Hund-Rinke, Fraunhofer IME
Status: in progress
Year of publication or finishing: available May 2009
Method: The concentration TiO2 will be dispersed in algae test medium (prepared according to the guideline).
Sample preparation method : Dispersion will be achieved by ultrasound and stirring; method will be optimized. After homogenization an appropriate number of algae will be added. Total concentration will change marginally. Agglomeration state will be measured at the test start.
Concentration of test substance: 50 mg/L; 25 mg/L; 12.5 mg/L; 6025 mg/L; 3.1 mg/L
Test organism: green algae
Results:
Comments :

3. Toxicity to Daphnia - Acute test

P 25:

Country / Organisation: Germany
Responsible: Fraunhofer IME, Kerstin Hund-Rinke: –
Year of publication or finishing:
Status: published,
Method: TiO2 was dispersed (with ultrasound) in daphnids test water
Sample preparation method : To avoid separation, the mixture was continuously stirred on a magnetic stirrer. The dispersion (50 mL in a 100 mL beaker) was irradiated (250 - 500 W; 15 - 30 min), in an Exposure System with simulated sunlight (300–800 nm). test performed in microtiter platesConcentration of test substance: 50 mg/L; 25 mg/L; 12.5 mg/L; 6025 mg/L; 3.1 mg/L
Test organism: Daphnia magna
Results:
Comments :

Country / Organisation: Germany
Responsible: BASF
Status: in progress
Year of publication or finishing:
Method: OECD 202
Sample preparation method
Test organism:
Results: informations will be provided later
Comments particle morphology (TEM, REM), particle size distribution
Density, apparent density, BET surface, Zeta Potential, purity, aggregate size distribution was assessed by analytical ultracentrifugation

Country / Organisation: Canada
Responsible: Trent University
Status: planned
Year of publication or finishing:
Method: OECD 202, acute and subacute
Sample preparation method
Test organism:
Results:
Comments :

Country / Organisation: Spain
Responsible: INIA
Status: planned
Year of publication or finishing:
Method: OECD 202
Sample preparation method
Test organism: Daphnia magna
Results:
Comments

Material 2, 3,4

Country-Organisation : Germany/BASF SE
Responsible:
Status:, accomplished
Year : 2006
Method: Short-term toxicity to aquatic invertebrates
Method : equivalent or similar to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)
Sample preparation method
Test organism: Daphnia Magna
Results: tables of different experiments without any conclusion
Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)

Material 12

Country / Organisation: BIAC
Responsible: DuPont, Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: OECD 202 - Static, Acute, 48-Hour Toxicity Screening Tests with Daphnia magna.
Test organism: water flea, Daphnia magna
Sample preparation method:
Results:
The acute toxicity of fine (pigmentary) or DLS (uf-C) TiO2 particles to the water flea, D. magna (less than 24 hold) was determined in unaerated, 48 h static tests according to OECD 202 testing guidelines (OECD, 2004).
The study was conducted with four concentrations each of fine or ultrafine TiO2 particles and a dilution water control at a mean temperature of 20.2 ◦C (range of 20.1–20.3 ◦C) and 20.1 ◦C (range of 20.0–20.2 ◦C), respectively. One test chamber was used per test substance concentration for each test substance with 10 test organisms in each chamber. Based on visual observations, the dilution water controls, 0.1, and 1.0 mg/L test concentrations were clear and colorless with no precipitate at test start. The 10 and 100 mg/L test concentrations were cloudy (white in color) and had suspended substance present at test start. All water quality parameters were within acceptable limits during the exposure.
Exposure of daphnids to the dilution water control and nominal fine (pigmentary) or ultrafine DLS (uf-C) TiO2 particle concentrations of 0.1, 1.0, 10, and 100 mg/L resulted in 0, 0, 0, 10, and 10% or 0, 0, 0, 10, and 0% immobility, respectively, at the end of 48 hours. The Daphnia magna 48 hour EC50 values for fine and ultrafine TiO2 particles, based on nominal concentrations were > 100 mg/L.
The results demonstrated that fine (pigmentary) or ultrafine DLS (uf-C) TiO2 particles exhibited low concern for aquatic hazard in unaerated, 48-hour, static acute tests.
Comments :
Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

4. Toxicity to Daphnia- *Reproduction Test*

P 25:

Country / Organisation: Canada
Responsible: Trent University
Status: in progress
Year of publication or finishing:
Method:
Sample preparation method
Test organism:
Results:
Comments

Country / Organisation: Spain
Responsible: INIA
Status: planned
Year of publication or finishing:
Method:
Reproduction test (OECD 211 Test Guideline) The use of K2TiO(C2O4)2·2H2O is under discussion.
Sample preparation method
Test organism: Daphnia magna
Results:
Comments :

Material 3

Country-Organisation : Germany/BASF SE
Responsible:
Status:, accomplished
Year : 2006
Method: A Daphnia reproduction test was performed according to the OECD guideline 211 with the test compound T-Lite™ SF-S.
Sample preparation method : The tested nominal concentrations were 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 mg l-1 plus control without test compound. Abiotic exposure conditions were the same as described for the acute toxicity tests. The semi-static exposure was conducted in 100 ml glass beakers filled with 50 ml test dispersion that were renewed three times per week. At each renewal oxygen content and pH were measured in one replicate per control and test concentration level in the old and in the fresh medium. Ten individually exposed daphnids per concentration level, aged less than 24 h at the start of the test, were fed daily with the green algae Desmodesmus subspicatus. The algae were separated from their culture medium by centrifugation, resuspended in M4 medium and stored in the dark at 4 to 8°C for a maximum of 21 days. By feeding the daphnids with the concentrated algae cell suspension the test dispersions were diluted by a maximum of 1.8% until the next medium exchange. During the test period of 21 days the individual food ratio was increased from 0.22 to 0.6 mg COD (chemical oxygen demand) per daphnid and day. Immobilization of the parent animals and offspring production was assessed daily. Neonates were counted and removed from the test vessels daily after appearance of the first brood..
Test organism: Daphnia magna
Results: No mortality of adults occurred in the controls over the whole testing period of 21 days. The mean number of live offspring produced per control parent animal surviving at the end of the test was 129 and the minimum number was 95, thus meeting the validity criterion according to the OECD guideline 211. During the 21 days of exposure to T-Lite" SF-S a concentration-response relationship for the number of live offspring per surviving female was found . The lowest-observed effect concentration (LOEC) was determined to be 10 mg l^{-1} resulting in a no-observed -effect concentration (NOEC) of 3 mg l^{-1}. Further, the first production of offspring was delayed by two days at e 10 mg/L . At 100 mg l^{-1} the surviving parental daphnids only produced a small number of offspring during the last four days of the exposure period. Mortality of parental animals occurred only at the highest concentration level (100 mg l^{-1}) where 6 of 10 daphnids were dead at the end of the test. No mortality occurred at the other test concentration levels or in the controls. Hence, a LOEC of 100 mg l^{-1} and a NOEC of 30 mg l^{-1} could be determined for the parameter mortality and the EC10 and EC50 for reproductive effects caused by T-Lite" SF-S during the chronic study were 5 and 26.6 mg l^{-1}, respectively
Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)

5. Toxicity to Fish

P 25:

Country / Organisation: Canada
Responsible: Trent University
Status: in progress
Year of publication or finishing:
Method: Early-life Stage Toxicity Test – modified, Developed protocol for dispersing material in water using sonication and centrifugation, Concentration of test substance: 0.1 to 10 ppm, Embryo rearing medium prepared from dechlorinated tap water (hard, high DOM).
Sample preparation method
Test organism: Medaka embryo/larval
Results:
Comments :

Country / Organisation: Spain
Responsible: INIA
Status: planned
Year of publication or finishing:
Method:
OECD 210 / OECD 212 test guidelines, modified, The use of K2TiO(C2O4)2·2H2O is under discussion, Embryo Rearing Medium (modified as necessary)
Sample preparation method
Test organism: Medaka embryo/larval exposure
Results:
Comments :

Country / Organisation: Spain
Responsible: INIA
Status: planned
Year of publication or finishing:
Method: Embryotest
It is intended to use a variety of endpoints related with oxidative stress, with cell membrane damage, and with cell detoxification pathways.
Sample preparation method
Test organism:
Results:
Comments :

Country / Organisation: US EPA
Responsible: Steve Diamond, Mid Continent Ecology Division
Status: in progress / planned
Year of publication or finishing:
Method:
OECD 210 Fish, Early-life Stage Toxicity Test – modified
Sample preparation method:

Currently developing methods for dispersing material in water that are consistent and repeatable - also adding organic matter as treatment. Sample preparation method under development, focusing on stirring first, avoiding solvents, or dispersants, sonicating as potential treatment. Test media: Moderately hard reconstituted water, MHRW with dissolved organic matter In exposure media are measured: Particle size (DLS, SEM), zeta potential, bulk concentration. Under development, focusing on stirring first, avoiding solvents or dispersants, sonicating as potential treatment

Test organism:
Results:
Comments

Country / Organisation Canada:
Responsible: *Richard D. Handy*
Status: published
Year of publication or finishing: 2007
Method:
Juvenile rainbow trout (n=189) were obtained from Hatchlands Trout Farm, Rattery, Devon, and held for 4 weeks in stock aquaria with flowing, aerated, dechlorinated Plymouth tap water. Stock animals were fed to satiation on a commercial trout food. Fish weighing 28.1±0.4 g (mean±S.E.M., n = 189) were then graded into twelve experimental glass aquaria (14 fish/tank), in a triplicate design (three tanks/treatment), and allowed to rest for 24 h prior to the commencement of the experiment. Fish were exposed in triplicate to one of the following treatments for 14 days using a semi-static exposure regime (80% water change every 12 h with re-dosing after each change): control (freshwater only), 0.1, 0.5 or 1.0 mg l−1 titanium dioxide nanoparticles (TiO2 NPs, see below for stock solutions). These concentrations of TiO2 NPs were selected after considering the concentrations used to produce epithelial injury and oxidative stress in rodents
Sample preparation method:
Test organism: Oncorhynchus mykiss
Results:
Aqueous exposure to TiO2 NPs did not cause mortality. Two fish died during the experimental period: one control fishwas lost due to fin nipping/aggression, and one fish exposed to 1.0 mg l−1 TiO2 at day 6 of exposure. The latter fish had signs of mucus secretion on the gills, consistent with gill pathologies.
In addition, some fish at the highest TiO2 concentration showed loss of position holding in the water column for brief periods towards the end of the experiment (hanging vertically in the water column for a few seconds–minute) which was indicative of fatigue or abnormal buoyancy control. No other unusual behaviours were observed.
Histological examination of the gills at the end of the experiment showed normal anatomy in the freshwater controls, with a normal background incidence of injuries on <5% of the secondary lamellae (<5% of filaments with swollen tips, <1% of the secondary lamellae showing any signs of oedema at the base, aneurisms and hyperplasia completely absent). Exposure to TiO2 NPs resulted in some increases in the incidence of oedema in the secondary lamellae, changes in mucocyte morphology, and hyperplasia in the primary lamellae. The proportions of secondary lamellae with oedema were (mean percentage± S.E.M., n = 6 fish): 0.7±0.7, 15.6±7.4, 5.7±10.0 and 5.0±1.0% of secondary lamellae for control, 0.1, 0.5 and 1.0 mg l−1 TiO2 NPs, respectively (0.1 mg l−1 TiO2 NPs was significantly higher than the control, t-test, $P < 0.05$). The TiO2 also showed a small but higher incidence of aneurisms on the secondary lamellae (absent in the controls).
Comments :
Aquatic Toxicology 84 (2007) 415–430. Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): Gill injury, oxidative stress, and other physiological effects
Gillian Federici, Benjamin J. Shaw, Richard D. Handy

Material 10

Country / Organisation: Canada
Responsible: Trent University
Status: in progress
Year of publication or finishing:
Method:
Acute and subacute toxicity to fish (Medaka embryo/larval exposure) Early-life Stage Toxicity Test – modified, Developed protocol for dispersing material in water using sonication and centrifugation, Concentration of test substance: 0.1 to 10 ppm, Embryo rearing medium prepared from dechlorinated tap water (hard, high DOM).
Sample preparation method
Test organism: Medaka embryo/larval
Results:
Comments

Material 12:

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: OECD 203 - The acute toxicity of fine (pigmentary) and DLS prototype (uf-C) TiO2 particle-types to the rainbow trout, Oncorhynchus mykiss was determined in unaerated, 96-hour static tests according to OECD 203 testing guidelines (OECD, 1992).
Sample preparation method The study was conducted with 4 concentrations each of fine-sized (pigmentary) rutile TiO_2 particles and ultrafine TiO_2 particles (DLS) and a dilution water control at a mean temperature of 12.2 ^oC (range of 12.1-12.3 ^oC) and 12.2 ^oC (range of 12.1-12.5 ^oC), respectively. One test chamber was used per test substance concentration for each test substance with 5 test organisms in each chamber. Based on visual observations, the dilution water controls, 0.1 mg/L and 1.0 mg/L test concentrations were clear and colorless with no precipitate at test start. The 10 and 100 mg/L test concentrations were cloudy with a slight amount of suspended substance present at test start. All water quality parameters were within acceptable limits during the exposure.
Exposure of rainbow trout to a dilution water control and nominal fine (pigmentary) TiO_2 and DLS (uf-C) TiO_2 particle concentrations of 0.1, 1.0, 10, and 100 mg/L resulted in 0, 0, 0, 10, and 10% or 0, 0, 0, 0, and 0% immobility, respectively, at the end of 96 hours.
The 96 hour LC_{50} for both types of TiO_2 particles was > 100mg/L based on nominal test concentrations
Test organism: Oncorhynchus mykiss (Rainbow Trout)
Results:
The results demonstrated that fine (pigmentary) TiO_2 and DLS (uf-C) each exhibited low concern (Smrchek et al., 1993) for aquatic hazard in unaerated, 96-hour, static acute tests.
Comments : Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

Other materials :
Literature: Federici et al., Toxicity of titanium dioxide nanoparticles to rainbow trout

Country / Organisation: Denmark
Responsible:
Status: published
Year of publication or finishing:
Method:
Sample preparation method
Test organism:
Results:
Comments : Data will be completed later

6. Effects on sediment species (short term/long term)
   This endpoint will be addressed by Germany, University of Bremen

7. Effects on soil species (short term/long term)

This endpoint will also be addressed by Germany, University of Bremen

Material ***8,11***

Country / Organisation: Canada
Responsible: MC Gill
Status: planned
Year of publication or finishing:
Method:
14 d-Acute lethality to earthworms, multi-generation test. Biological Test Method: Tests for toxicity of contaminated soil to earthworms (Env Canada, EPS 1/RM/43, 2007)
Sample preparation method : Developing methods for soil spiking and determination of exposure dose. Concentration of test substance: 1 to 10,000 mg/kg dry soil.
Test organism: Eisenia fetida
Results:
Comments :

8. Effects on terrestrial species

P 25, and other materials

Country-Organisation : France/ CEA Saclay
Responsible: Marie Carriere, Nathalie Herlin
Status: in progress
Year of publication or finishing :
Method: Phytotoxicity and accumulation in plants (Arabidopsis thaliana, Indian mustard)
TiO2 purchased from sigma, degussa and synthesized by laser pyrolysis in DSM/IRAMIS/SPAM laboratory (CEA Saclay)
Sample preparation method :.. cell culture media - hoagland medium
Test organism: Arabidopsis thaliana, Indian mustard
Results:
Comments :

Country / Organisation: Germany
Responsible: Umweltbundesamt
Status: planned
Year of publication or finishing: 2010
Method: according to the results from the OECD sponsorship programme
Sample preparation method *Test organism:*
Results:
Comments :

9. Effects on microorganisms

P 25, and other materialss

Country-Organisation : France/ CEA Saclay
Responsible: Marie Carriere, Nathalie Herlin
Status: in progress
Year of publication or finishing :
Method: acute toxicity, accumulation, surface modification by bacteria (Cupriavidus metallidurans CH34, E. Coli MG1655)
TiO2 purchased from sigma, degussa and synthesized by laser pyrolysis in DSM/IRAMIS/SPAM laboratory (CEA Saclay)
Sample preparation method cell culture media - hoagland medium
Test organism: Cupriavidus metallidurans CH34, E. Coli MG1655
Results:
Comments :

Country / Organisation: Germany
Responsible: Umweltbundesamt and other
Status: planned
Year of publication or finishing: 2010
Method: according to the results from the OECD sponsorship programme
Sample preparation method
Test organism:
Results:
Comments

10. Other relevant endpoints

P 25

Country / Organisation: Spain
Responsible: INIA
Status: planned
Year of publication or finishing:
Method:
Ecological effects on plankton communities. Guidance document on simulated freshwater lentic field tests (outdoor microcosm and mesocosm) OECD Series on Testing and Assessment No. 53
Sample preparation method
Test organism:
Results:
Comments

Country / Organisation: Defra
Responsible: Richard Handy - John Garrod, Defra
Status: planned
Year of publication or finishing:
Metihod:

(i) oxidative stress and the generation of reactive oxygen species
(ii) immunology- maybe it causes inflammation in fish
(iii) DNA damage or genotoxicity in wildlife.

Sample preparation method
Test organism:
Results:
Comments :

Mammalian toxicology

1. Pharmacokinetics/toxicokinetics (ADME)

P 25:

Country / Organisation: Germany
Responsible: Fraunhofer ITEM
Status: in progress
Year of publication or finishing:
Method: adapted OECD 412 adapted OECD 417; combination of 3-wk inhalation test with toxicokinetics; generally: more endpoints than requested by guideline to address nanoparticle-specific issues (migration, translocation, effects on remote organs); Haematology, bronchoalveolar lavage (BAL), urinalysis, histopathology, cell proliferation, SEM/TEM analysis, target organs: respiratory tract and brain;
Sample preparation method TiO2-suspension in phosphate-buffered solution; vortexing and ultrasonic treatment before nebulizing the particle suspension; Concentration of test substance: 0.1% titanium dioxide P 25 in 0.15% phosphate buffer (suspension for nebulization; aerosol concentrations of 2 and 5 mg/m3 P-25 for exposure to rats, Reference substance: titanium dioxide Bayertitan T (fine-fraction, BAYER AG), Characterization of test material in exposure media. Agglomeration during the test: (particle size distribution determined at consecutive time-points to assure that P 25 in suspension was nanoscaled throughout the exposure period (particle suspension for nebulization);
Test organism: rat
Results:
Comments :

Country / Organisation: USA
Responsible:
Status:
Year of publication or finishing:
Method:
Sample preparation method
Test organism:
Results :
Comments ::
An FDA study should be available; The National Toxicology Program (NTP) is also conducting mammalian toxicity tests with Degussa P25, point of contact: Nigel Walker, NTP, Research Triangle Park, NC.

Country / Organisation: Germany
Responsible: Helmholtz Zentrum München
Status: in progress
Year of publication or finishing:

Method: Using quantitative bio-kinetic analysis of radiolabeled inhaled TiO_2 particles the retained part in selected organs and tissue including excrements will be investigated. Healthy adult Wistar Kyoto rats of both genders are exposed by intratracheal inhalation to vanadium V-48-radiolabeled TiO_2 nanoparticles. A complete V-48 balance of all organs, tissues, excretion and remaining carcass will be performed 1h, 24h, 1 week and 1 month after application in rat.
Sample preparation method:
Results:
Comments :

Material P25?-
NOTE BASF said they were using P25 but :in their document the TiO2 25 uncoated is a mixture anatase/rutile 70:30
Country-Organisation : Germany/BASF SE
Responsible:
*Status:*publised
Year of publication or finishing : 2008
Method: equivalent or similar to OECD Guideline 417 (Toxicokinetics)
Sample preparation method single intravenous injections of a suspension of 0.5% TiO2 in serum. This dose was chosen because it was high enough to allow spectroscopic quantification of residue levels in organs of interest (based on estimated calculations of residue levels with a distribution volume of 1 and a known limit of quantitation (loq) of the atomic absorption method used) and low enough to be acutely nontoxic (determined in a subgroup of animals by dosing with 5 mg/kg and observing no visible symptoms of toxicity for 1 day).
PHARMACOKINETIC STUDY (Absorption, distribution, excretion)

- Tissues and body fluids sampled: blood, plasma, kidney, spleen, brain, lymph nodes (mediastenal, mesenteric, and popliotenal), liver, and lung
- Time and frequency of sampling: 1, 14 and 28 days after application
- Method for quantification: ICP-AES (limit 0.5 µg/tissue)
  OTHER:
- In addition to TiO2 tissue distribution, various cytokines and enzymes (a total of 67 parameters) were measured in blood samples to assess potential inXammatory responses and/or organ injury due to intravenousexposure to TiO2. These parameters were measured at Rules-Based Medicine, Inc. (Austin, TX, USA).

Test organism: Male Witar Rat
Results: There were no detectable levels of TiO2 (<0.5 µg/organ) in blood cells, plasma, brain or lymph nodes (mediastenal, mesenteric, and popliotenal) at any of the three time points tested. The distribution of TiO2 in liver, spleen, lung, and kidney of control animals and experimental animals are shown in table below. The TiO2 levels were highest in the liver, followed in decreasing order by the levels in the spleen, lung, and kidney (very low levels of <0.7 µg). In all four organs, TiO2 levels were highest on day 1. TiO2 levels were retained in the liver for the 28-day duration of the experiment with the exception of the results for one experimental animal on day 14, which had a noticeably lower level of TiO2 (35.6 µg/g) in the liver than the other two experimental animals at day 14 (138.8 and 124.2 µg/g). In the spleen, the results were more variable than in the liver, but showed a trend of a slight decrease in TiO2 levels from day 1 to days 14 and 28. An early distribution of TiO2 to the lung on day 1 was seen with a return to near control levels by day 14. Similarly, in the kidney, there was an increase in TiO2 levels compared with control on day 1 and a return to control levels by day 14.
Remark : None of the examined cytokines and chemokines showed definitive changes at days 1, 14, or 28 following TiO2 exposure, indicating that there was no detectable inflammatory response or organ toxicity

Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)
Fabian *et al.*, Arch Toxicol, 2008, 82(3):151-7

Material 2, 3,

Country-Organisation : Germany/BASF SE
Responsible:
Status: published
Year of publication or finishing : 2006
Method: OECD Guideline 428 (Skin Absorption: In Vitro Method)
Sample preparation method The dermal penetration in vitro of Titanium (Ti) from a Titanium Dioxide containing cosmetic formulation (emulsion with T-Lite SF-S (TiO2) 10 %)through dermatomed pigskin (thickness 400 - 700 µm) was assessed by single topical application of a target dose of about 4 mg per cm² of the test emulsion (corresponding to approximately 400 µg/cm² TiO2 or 240 µg/cm² Ti) to pigskin preparations mounted on Franz-type diffusion cells.

Skin of 3 domestic pigs was used. Three dermatomed skin preparations of each pig were treated with the TiO2 containing formulation. One untreated skin preparation of each pig was analyzed to establish the absence of Ti in the skin samples.

Diffusion cells were operated in the static mode with physiological saline containing 5% bovine serum albumin (BSA) as the receptor fluid. The openings of the donor compartments were covered with Fixomull Stretch adhesive fleece (semi-occlusive conditions) after application. During the 24-hour exposure period, samples of the receptor fluid were collected from each cell at several time points in order to determine the cumulative absorption of Ti to the receptor fluid. At the end of the exposure period the test substance was removed from the skin preparations by washings with sponge pieces dipped into a soap solution. Tape stripping (10 Scotch tapes pooled into 2 samples of 5 tapes, each) was used to remove Ti together with the superficial layers of the stratum corneum. Furthermore, Ti was recovered from the skin preparation and all other relevant compartments of each diffusion cell. The results of recovery are summarized as non-absorbed dose (washing, tape stripping), amount associated to the skin preparation and absorbed dose (amount present in the receptor fluid).
Test organism: pig
Results:
ABSORPTION IN DIFFERENT MATRICES

- Skin wash: Virtually the total amount of applied Ti was removed from the skin surface by the washing procedure.
- Receptor fluid, receptor chamber, donor chamber (in vitro test system): No Ti was found in the receptor fluid at any sampling time and thus no penetration of Ti ions or TiO2 particles occurred.
- Skin preparation (in vitro test system): The amounts of Ti found in the skin preparations were in the range of the analytical determination limit (0.3 µg total Ti in the sample).
- Stratum corneum (in vitro test system): The amounts of Ti found in the tape strips were in the range of the analytical determination limit (0.3 µg total Ti in the sample).

TOTAL RECOVERY

- Material 2 : The mean total recoveries of Ti measured in diffusion cells equipped with skin of the 3 pigs were in the range of 86 – 93 % and thus fulfill the SCCNFP and with the exception of pig 3 also the OECD quality criteria.

- Material 3:The mean total recoveries of Ti measured in diffusion cells equipped with skin of the 3 pigs were in the range of 98 – 100 % and thus fulfill the SCCNFP and OECD quality criteria.

In conclusion, the amount of Ti applied to the skin preparations as TiO2 containing cosmetic formulation was quantitatively removed by skin washing. The tiny quantities of Ti in the tape strips and skin membranes in the magnitude of the analytical limit of determination may stem from furrows or hair shaft openings in the skin, which were not fully accessed by the washing and tape stripping procedure. There was no increase of Ti in the receptor fluid.
Thus no Ti from the TiO2 containing formulation penetrated into or through the skin under the conditions of this study. In addition these results show that micro fine TiO2 particles are not able to penetrate the porcine dermatomed skin preparations.
Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)
Gamer *et al.* Toxicology in Vitro, 2006, 20: 301-307

Material:18
Country-Organisation : European comission
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)
*Status:*published
*Year of publication or finishing :*2000
Method: "An investigation into the absorption of the active ingredient (a.i.) was carried out in pig skin in vitro, using Franz cells; the skin was thereafter stripped and the distribution of the a.i. studied by electron micrography.
Sample preparation method For the first part of the investigation, the skin was placed in the cells, with an exposed area of approximately 5 cm2. The temperature was maintained at 32o. The a.i. was formulated in an oil in water emulsion, containing 4% T805®. This is a coated preparation with a mean diameter of 20 nm, although electron micrography at a later stage of the experiment showed formation of agglomerates. The receptor fluid was saline with gentamicin and 1% bovine serum albumin. The application was at a rate of 4 mg of formulation per square centimetre. The cells were allowed to stand for 24 hours. Thereafter, the skin was cut into small areas by use of a punch and prepared for electron microscopy (both transmission and scanning); the identification of titanium in the sections was by energy dispersive X-ray analysis (EDXA) using a titanium-specific energy window.
Test organism: Pig skin, Franz cells
Results:. Surprisingly, no chemical investigation of the receptor fluid is reported. The authors studied the distribution of the a.i. by electron micrography. The a.i. was confined to the stratum corneum. It penetrated the outer portions of the hair follicles in minute amounts, but was not found in any of the cells lining the follicle. [No mention is made of sweat glands; this may be because of anatomical differences between pig and human skin]. Repeated stripping suggested that the a.i. was found initially on the ridges of the skin surface; as stripping continued, there was access to the material found in the furrows. The authors suggest that this gradually declining concentration of the a.i. with successive strippings is not to be interpreted as a penetration of the a.i. into the stratum corneum, but rather to a differential sampling of the skin, so that the early strippings, taking up the material on the ridges of the skin, show a higher amount of a.i. than the later strippings, which are derived from the furrows, and which contain a smaller amount of the a.i.
Comments: A study was also carried out in vivo on human forearm, which is stated to have confirmed the above findings, but this study is not reported in the present SCCNFP submission.

Opinion of the Scientific Committee on Cosmetic Products and non-food products intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. F. Pflücker, H, Hohenberg, E Hölzle et al. The outermost stratum corneum layer is an effective barrier against dermal uptake of topically applied micronized titanium dioxide, J Invest Dermatol (1999), 399-411

Country-Organisation : European comission
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)
*Status:*published
*Year of publication or finishing :*2000
Method: " The experiments were performed according to GLP. The test substance was T805.
Sample preparation method The formulation contained 5% of the active ingredient. Skin samples were obtained post mortem from 3 female donors and one male. The ages of the donors ranged from 23 to 70 years. Fat was removed from the samples, and they were stored at -20°C until required. Before the experiment, the skin was allowed to thaw, and the sample immersed in water at 60°C for a minute The epidermis was then removed with a forceps, and mounted in a cell; the formulation was applied at a rate of about 3.6 mg/cm². The area of the exposed skin was 0.32 cm². The receptor fluid was physiological saline with tetracycline hydrochloride 1 µg/ml, and was perfused at a rate of 1.5 ml.h-1. The integrity of the membrane was checked by the use of tritiated water and calculation of the permeability coefficient for each skin. Skins with coefficients greater than 1.5 X 10-3 cm. h-1 were excluded. The diffusion was allowed to proceed for 8 hours. Following the diffusion process, the skins were fixed for electron microscopy.
The active ingredient was looked for in the receptor fluid using inductive coupled plasma mass spectrometry. This technique involves the introduction of the samples of receptor fluid as a spray into inductively coupled argon gas plasma, which converts (e.g.) titanium into an ion and allows quantitation according to the mass/charge quotient. The method gives a result linearly related to the concentration of the analyte in the sample, and the limit of detection is 1.5 ng/ml in the original sample. The skin was also examined by transmission electron microscopy.
Test organism: Human Skin sample
Results: No titanium dioxide was found in the receptor fluid within the limits of detection. Using electron microscopy, the titanium dioxide was found to be confined to the outer layers of the stratum corneum. It was concluded that the active ingredient was not absorbed by human skin ex vitro.
Comments: Opinion of the Scientific Committee on Cosmetic Products and non-food products intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. Degussa AG, US-IT No. 94-0158-DGT. 1996. The in-vitro percutaneous absorption through human abdominal epidermis of titanium dioxide from titanium dioxide T805 formulation.

Other material:
France: in progress

2. Acute Toxicity - Inhalation

P25

Country / Organisation: Germany
Responsible: Fraunhofer ITEM (Otto Creutzenberg)
Status: completed
Year of publication or finishing:
Method: intratracheale Instillation adapted OECD 403/433 intratracheal instillation instead of acute inhalation,
Sample preparation method:TiO2-suspension in PBS-buffered saline + Detergent Tween; vortexing and ultrasonic treatment before administration to rats; 2 mg per 0.4 ml administration buffer Characterization of test material: producer and own characterization Test material: PBS-saline/1% Tween 80
Test organism: rat
Results:
Comments :

Country / Organisation: Germany
Responsible: Fraunhofer ITEM (Jens Hohlfeld)
Status: in Progress
Year of publication or finishing:
Method: influence on pulmonary surfactant ultrastructure, adapted OECD 403/433
Sample preparation method: P 25 as nanoscaled particles in suspension detected (ZetaSizer); TiO2-suspension in phosphate-buffered solution; vortexing and ultrasonic treatment before nebulizing the particle suspension; 0.1% titanium dioxide P 25 in 0.15% phosphate buffer (suspension for nebulization); aerosol concentration of 10 mg/m3 P-25 for exposure to rats; reference material: titanium dioxide Bayertitan T (fine-fraction, BAYER AG)
Test organism rat
Results:
Comments

Country / Organisation: Germany
Responsible: NanoCare
Status: in Progress
Year of publication or finishing:
Method: Short-Term inhalation Test including lavage
Sample preparation method: Lung lavage, with recovery period, particle morphology (TEM), 5-day Study
Sample preparation method:
Results:
Comments : including particle size distribution, density, apparent density, BET surface, Zeta Potential, purity.

Material 1:

Country / Organisation: Switzerland/Germany
Responsible:
Status: in Progress
Year of publication or finishing:
Method:

1) inhalation of anesthetized, intubated rats via computer controlled breathing in a whole body plethysomograph; morphometric investigation of lung tissue and aerosol sample grids with with a LEO 912 transmission electron microscope equipped with an energy filter for elemental microanalysis; detectable lung toxicity determined by ultrastructural TEM analysis; adult healthy male WKY rats;
2) inhalation of anesthetized, intubated rats via computer controlled breathing in a whole body plethysomograph; morphometric investigation of lung tissue and aerosol sample grids with with a LEO 912 transmission electron microscope equipped with an energy filter for elemental microanalysis;
3) inhalation of anesthetized, intubated rats via computer controlled breathing in a whole body plethysomograph; After aerosol exposure, rats were immediately killed and subjected to BAL. Morphometric investigation of BAL cells and aerosol sample grids with with a LEO 912 transmission electron microscope equipped with an energy filter for elemental microanalysis was done;

Sample preparation method
Test organism: rat
Results:
Representative TEM picture(s) yes, many from aerosol as well as in lung tissue as a result of the morphometric study, Particle size distribution, CMD 22 nm, GSD 1.7; also size disrtribution in lung tissue: CMD 29 nm, GSD 1.7; Specific surface area: 300 m²/g; Zeta potential (surface charge); particle profiles in the lungs by TEM+EELS (electron-energy-loss-spectroscopy): 93% of the particles investigated in the ultrathin lung sections had a round or oval compact shape rather than the original chain aggregated/agglomerated structure, whereas 7% were needle-like; particles in the lung tissue had a count median diameter of 29 nm and a geometric standard defiation of 1.7; inhalation of anesthetized, intubated rats via computer controlled breathing in a whole body plethysomograph; morphometric investigation of lung tissue and aerosol sample grids with with a LEO 912 transmission electron microscope equipped with an energy filter for elemental microanalysis; Aerosols of ultrafine TiO2 particles were generated with a spark generator (GFG 1000, Palas Karlsruhe, Germany) in a pure argon plus 0.1% oxygen stream (Roth et al., 2004); formation of 2-4 nm primary particles which aggregated / agglomerated while cooling in the gas; agglomeration was stopped by gas dilution with nitrogen and oxygen to simulate synthetic air during conditioning and humidifying of the aerosol; dose: 4-5 µg inhaled TiO2 in each animal. Media: synthetic air; the aerosol in argon was diluted and conditioned by adding oxygen and nitrogen to achieve 21% oxygen balanced by nitrogen, as well as by humidifying it to 60–70% relative humidity and warming it up to 37°C; Three studies with the same procedure: no detectable lung toxicity determined by ultrastructural TEM analysis and cell differentiation of BAL cells; no inflammatory cell responses observed upon the inhalation of ultrafine TiO2 particles in the animal groups
C*omments:*

Material 6

Country / Organisation: Germany
Responsible: Fraunhofer ITEM
Status: completed
Year of publication or finishing:
Method:
intratracheale
Sample preparation method
Instillation, TiO2-suspension in PBS-buffered saline + Detergent Tween; vortexing and ultrasonic treatment before administration to rats; 2 mg per 0.4 ml administration buffer
Characterization of test material: producer and own characterization
Test material: PBS-saline/1% Tween 80
Test organism: rat
Results:
Comments :

Material 12:

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: Short term pulmonary toxicity study via instillation
Sample preparation method
Test organism: rats
Results:
The aim of this study was to assess lung toxicity in rats of newly developed, well characterized, ultrafine-TiO2 particles and compare them to TiO2 samples in two different size ranges and surface modifications.
The ranking of lung inflammation/cytotoxicity/cell proliferation and histopathological responses (in descending order) was quartz > 80:20 anatase:rutile uf TiO2 > fine-sized TiO2 = uf-A = uf-B where uf-A and uf-B were DLS. Exposures to quartz and to a lesser degree, 80:20 anatase:rutile TiO2 particles produced pulmonary inflammation, cytotoxicity and adverse lung tissue effects. In contrast, exposures to fine-TiO2 particles or to rutile uf-A/uf-B TiO2 particle-types produced transient inflammation and no adverse histopathological or cell proliferative effects in the lungs of exposed rats at any post exposure time period. It was concluded that differences in responses to 80:20 anatase:rutile TiO2 particles vs. the rutile uf-A and uf-B (DLS) TiO2 particle-types could be related to crystal structure, inherent pH of the particles, or surface chemical reactivity. Finally, the results demonstrate that exposures to ultrafine-TiO2 particle-types can produce differential pulmonary effects, based upon their composition, crystal structure and/or surface reactivity.
Comments: Warheit DB, Webb TR, Reed KL, Frerichs S, and Sayes CM. Pulmonary Toxicity Study in Rats with Three Forms of ultrafine-TiO2 Particles: Differential Responses related to Surface Properties. Toxicology 230:90-104, 2007.

3. Acute Toxicity - Oral route

Material 12:

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: OECD 425 guidelines and US EPA (OECD, 2001;USEPA, 2002) - Acute Oral Toxicity Study in Rats - Up and Down Procedure
Sample preparation method
Single dose of uf-C (DLS) TiO2 particles suspended in deionized water was administered by oral gavage to one fasted female rat each at a dose of 175, 550, or 1750 mg/kg and to three fasted female rats at a dose of 5000 mg/kg. The rats were dosed one at a time at a minimum of 48-hour intervals. The rats were observed for mortality, body weight effects, and clinical signs for 14 days after dosing. All rats were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction.
Test organism: rats
Results:
No mortality occurred on the study. The single rat dosed at 1750 mg/kg and the three rats dosed at 5000 mg/kg exhibited grey colored feces during the study. No biologically important body weight losses occurred. No gross lesions were present in the rats at necropsy.
Based on this study, the oral LD50 for uf-C TiO2 particles was greater than 5000 mg/kg for female rats.
Comments: Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

Material 14,15,16

Country / Organisation: Denmark, NRCWE
Responsible:
Status: in progress
Year of publication or finishing:
Method: adapted OECD guidelines and other.s The mentioned repro and behavioral study is only completed on one of the TiO2 samples at this point.
Sample preparation method
Test organism:
Results:
Comments ::

Germany question : Also for participation with the listed samples we need to discuss whether our test materials should be distributed to other groups or the different members can obtain the “same” material from the suppliers.
France answer : This discussion already occurred in Busan meetings. The response was yes.
That the reason why France have the insurance from TDMA after asking :, samples will be provided to all participants.

Material 18
Country-Organisation : European comission
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)
*Status:*published
*Year of publication or finishing :*2000
Method: Titanium dioxide T805® was investigated according to GLP and in accordance with Council Directive 92/32/EEC.
Sample preparation method : The material, suspended in arachid oil, was given by gavage as a single dose of 2150 mg/kg bw to 10 rats (strain Hsd/Win:WU), 5 males and 5 females. There were no toxic effects, and no deaths. Observation was for 14 days. There was no effect on body weights. Gross necropsy revealed no abnormalities. The LD50 was > 2150 mg/kg bw.
Test organism: rat
Results: There were no toxic effects, and no deaths. Observation was for 14 days. There was no effect on body weights. Gross necropsy revealed no abnormalities. The LD50 was > 2150 mg/kg bw.
Comments: From Opinion of the Scientific Committee on Cosmetic Products and non-food products intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. Degussa AG, US-IT No. 93 -0060-DGT. 1993 :'Acute Toxicity - Testing the acute toxicity after single oral administration in rats

4. Acute Toxicity Dermal Route

some materials:
Germany: INM: Results from Project Nanoderm

5. Repeated dose toxicity - subacute

-Inhalation

P 25?
NOTE BASF said they were using P25 but :in their document the TiO2 25 uncoated is a mixture anatase/rutile 70:30

Country-Organisation : Germany/BASF SE
Responsible:
Status: published
*Year of publication or finishing :*2009
Method: Study similar to OECD guideline 412 with acceptable restrictions (non-GLP, short exposure period, histopathology restricted respiratory tract, mediastinal lymph nodes, brain with olfactory bulb). In addition examination of lung lavage, cell proliferation of the lung, lung burden, determination of titanium content in lung, lung associated lymph nodes, brain with olfactory bulb, liver, kidney, spleen and blood.
Sample preparation method : Male Wistar rats were exposed to aerosols of 0 (control), 2, 10 and 50 mg/m3 nano-titanium dioxide (TiO2) by inhalation for 6 h/day for 5 days. Necropsies were performed either immediately after the last exposure or after 3 or 16 days post exposure.
DETAILS ON INHALATION EXPOSURE
- generation of test atmosphere / chamber description
  Dust aerosols were produced at target concentrations of 2, 10 and 50 mg/m3 by dry dispersion of powder pellets with a brush dust generator (developed by the Technical University of Karlsruhe in cooperation with BASF, Germany). Each concentration was

generated with compressed air in a mixing stage, mixed with conditioned dilution air and passed via a cyclone (to separate particles > 3 µm) into a head-nose inhalation system. The different target concentrations were achieved by varying the feeding speed of the substance pellet and the rotating speed of the brush. To reduce electrostatic charging, brushes made of stainless steel were used. The generator itself and all conducting tubes were grounded.

- Temperature, humidity, pressure in air chamber: 22-24 °C, 50 %, 10 mbar
- Air flow rate: 6 m3/h
- Air change rate: 67
- Method of particle size determination: cascade impactor, optical particle spectrometer, scanning mobility particle sizer
- Treatment of exhaust air: filtering

- test atmosphere
  - Brief description of analytical method used: gravimetrical measurement
  - Samples taken from breathing zone: yes

DETAILS ON STUDY DESIGN
- Dose selection rationale:
- Rationale for selecting satellite groups: recovery
- Post-exposure recovery period in satellite groups: 3 and 16 days

Test organism: Male Wistar rats

Results:

CLINICAL SIGNS AND MORTALITY: no effects

BODY WEIGHT AND WEIGHT GAIN: no effects

BRONCHOALVEOLAR LAVAGE: The inhalation exposure to nano-TiO2 caused concentrationrelated increases in total cell counts, total protein content and enzyme activities. The increase in total cell counts was due to increased numbers of polymorphonuclear neutrophils (PMN), no significant effects of exposure on BALF eosinophil, lymphocyte and macrophage cell counts being observed. Three days after the last exposure (study day 8), minimal effects on total protein and some enzyme activities were also observed at the low concentration of 2 mg/m3 nano-TiO2. After the recovery period of 16 days (study day 21) most of these parameters returned to the control level, with only the PMN count and LDH activity in the high concentration group being significantly increased above control levels. The most pronounced effect was observed 3 days after the exposure.

ORGAN WEIGHTS: increased absolute lung weights in the high concentration group immediately after exposure

GROSS PATHOLOGY: no findings

HISTOPATHOLOGY: Immediately after exposure, diffuse histiocytosis and a mild neutrophilic inflammation was observed in the lungs of intermediate and high concentration animals. In the bronchioli and bronchi of the main group animals, mild hyperplasia/hypertrophy was observed. After the 16-day recovery period all treated animals still revealed pigment-loaded macrophages within the lungs. However, in four of the animals exposed to 10 mg/m3 nano-TiO2 and all animals exposed to 50 mg/m3 nano-TiO2 the distribution of the histiocytes was changed from a diffuse to a multifocal pattern. The other two animals of the 10 mg/m3 nano-TiO2 group revealed a minimal diffuse infiltration with histiocytes.

OTHER FINDINGS:

Titanium content in the tissues: In the test animals, Ti was only detectable in lung and mediastinal lymph nodes of the exposed animals. The detection limit for Ti was 0.3 µg per tissue, corresponding to 0.5 µg TiO2.

Cell proliferation: After five days of exposure to the different particles, all treated animals showed an increase in cell proliferation in terminal bronichioli and bronchi. The strongest increase was observed in the terminal bronchioli. Apoptosis was significantly increased in large/medium bronchi

and terminal bronchioli. The most pronounced effect was observed immediately after the exposure.
Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)
Ma Hock *et al.*, Inhalation Toxicology , 2009, 21 (2): 102-118

Material 2

Country-Organisation : Germany/BASF SE
Responsible:
Status: experimental result
*Year of publication or finishing :*2009
Method: Study similar to OECD guideline 412 with acceptable restrictions (non-GLP, short exposure period, histopathology restricted to respiratory tract, mediastinal lymph nodes, brain with olfactory bulb). In addition examination of lung lavage, lung burden, determination of titanium content in lung, lung associated lymph nodes, brain with olfactory bulb, liver, kidney, spleen and blood.

Sample preparation method : Male Wistar rats were exposed to aerosols of 0 (control), 0.5, 2 and 10 mg/m3 nano-titanium dioxide (TiO2) by inhalation for 6 h/day for 5 days. Necropsies were performed either immediately after the last exposure or after 3 or 24 days post exposure.
DETAILS ON INHALATION EXPOSURE

- Generation Of Test Atmosphere / Chamber Description
  Dust aerosols were produced at target concentrations of 0.5, 2 and 10 mg/m3 by dry dispersion of powder pellets with a brush dust generator (developed by the Technical University of Karlsruhe in cooperation with BASF, Germany). Each concentration was generated with compressed air in a mixing stage, mixed with conditioned dilution air and passed via a cyclone (to separate particles > 3 µm) into a head-nose inhalation system. The different target concentrations were achieved by varying the feeding speed of the substance pellet and the rotating speed of the brush. To reduce electrostatic charging, brushes made of stainless steel were used. The generator itself and all conducting tubes were grounded.
  - Temperature, humidity, pressure in air chamber: 22-24 °C, 50 %, 10 mbar
  - Air flow rate: 6 m3/h
  - Air change rate: 67
  - Method of particle size determination: cascade impactor, optical particle spectrometer, scanning mobility particle sizer
  - Treatment of exhaust air: filtering
- Test Atmosphere
  - Brief description of analytical method used: gravimetrical measurement
  - Samples taken from breathing zone: yes

DETAILS ON STUDY DESIGN

- Dose selection rationale:
- Rationale for selecting satellite groups: recovery
- Post-exposure recovery period in satellite groups: 3 and 24 days

Test organism: Male Wistar rats
Results:
Summary of the subtance-related adverse findings:

Group 3 (10 mg/m3):

- significantly increased protein concentration in the lavage fluid ($p < 0.05$)
- significantly increased acitivities of lactate dehydrogenase , gamma-glutamyltransferase,

alkaline phosphatise and N-acetyl-β-D-glucosaminidase in lavage fluid ($p < 0.01$)
- significantly increased total cell count, count of polymorphnuclear neutrophil granulocyte, and monocyte in the lavage fluide ($p < 0.01$)
- After a recovery period of 23 days, protein concentration ($p < 0.01$), the activities of lactate dehydrogenase ($p < 0.05$) and alkaline phosphotase ($p < 0.01$) in the BALF were still significantly increased, though less pronounced than 2 days after the exposure.

Group 2 (2 mg/m3)
- significantly increased acitivities of lactate dehydrogenase ($p < 0.05$) and alkaline phosphatise in lavage fluid ($p < 0.01$)
- significantly increased count of polymorphnuclear neutrophil granulocyte ($p < 0.01$) and monocyte ($p < 0.05$) in the lavage fluide

Group 1 (0.5 mg/m3)
- sNo substance-related adverse findings

Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)

Material 12:
BIAC, Du Pont: Short term Pulmonary Toxicity via instillation,

6. Repeated dose toxicity - subchronic

-Inhalation

P25
Country / Organisation: Germany
Responsible: Fraunhofer ITEM
Status: published
Year of publication or finishing:
Method: inhalation test. adapted OECD 452
Sample preparation method
Aerosolization of the dry P 25 powder (MMAD: 0.8 µm - GSD: 1.8); aerosol concentration of average 10 mg/m3 P-25 for exposure to rats/mice ;
exposure for 4.5 mths, 95 hrs/wk ;
Referenced material: carbon black, diesel exhaust ;
test medium: airborne
Test organism: rat
Results:
Comments : Agglomeration during the test: MMAD analysis, Additional endpoint: retention of test item in lungs and LALN; lung clearance measured using radio-tagged tracer

Country / Organisation: CEFIC
Responsible: ACC
Status: in Progress
Year of publication or finishing:
Method: in - vivo subchronic inhalation toxicity conducted at CIIT under CIIT Research Quality Standards (RQS) and partly under GLP
Sample preparation method: Animals were exposed by whole-body inhalation for 6 hours/day, 5

days/week over 13 weeks to target aerosol concentrations of 0.5, 2.0, or 10mg TiO2/m3 in 1 m3 H-1000 stainless steel chambers. Post exposure up to 52 weeks (rats, mice), to 49 weeks (hamsters). Time points of selected endpoint measurements: 0, 4, 13, 26, 52 (49) weeks post exposure. For aerosol generation a dust feeder (brush generator), jet streams air and a mixing chamber were used. Characteristics in exposure media: CIIT Research Center: Particle size analysis: Hamster average MMAD 1,29 µm SD 0.30, mouse average MMAD 1.45 µm SD 0.49, rat average MMAD 1.44 µm SD 0.57.
Test organism: Female B6C3F1 mice, female Fischer 344 rats and female Syrian Golden Hamster
Results:
Comments :

(A companion study was performed to examine the reponse of these rodents to the inhalation of pigmentary TiO2.)

P 25 and material 19
Country-Organisation : USA/*CIIT Centers for Health Research, Research Triangle Park, North Carolina,*
Responsible: Edilberto Bermudez
Status:, published
*Year of publication or finishing :*2004
Method: A multispecies, subchronic, inhalation study comparing pulmonary responses to ultrafine titanium dioxide (uf-TiO2) was performed.
Female rats, mice, and hamsters were exposed to aerosol concentrations of 0.5, 2.0, or 10 mg/m3 uf-TiO2 particles for 6 h/day, 5 days/week, for 13 weeks. Following the exposure period, animals were held for recovery periods of 4, 13, 26, or 52 weeks (49 weeks for the uf-TiO2–exposed hamsters) and, at each time point, uf-TiO2 burdens in the lung and lymph nodes and selected lung responses were examined. The responses studied were chosen to assess a variety of pulmonary parameters, including inflammation, cytotoxicity, lung cell proliferation, and histopathological alterations.
Sample preparation method:
Test organism: Female rats, mice, and hamsters
Results: In summary, inhalation of 10 mg/m3 uf-TiO2 for 13 weeks resulted in pulmonary overload in rats and mice but not in hamsters where the lung burdens were approximately 23% of the other species. While there were various responses in mice and rats, hamsters had very limited responses probably due to the low lung burdens and rapid clearance of particles in these animals. Responses in mice were limited to animals exposed to 10 mg/m3, whereas in rats responses were also observed in animals exposed to 2 mg/m3. The magnitude and spectrum of responses were, in general, equivalent in rats and mice. The extent and character of the inflammatory responses in rats differed from mice; in rats the responses had a greater neutrophilic component that diminished with time, whereas in mice significantly increased neutrophil and macrophage numbers remained relatively constant. Histopathological examination of rats and mice uncovered progressive fibroproliferative lesions in rats but not in mice. Taken together, the species differences observed in this study reflect the outcome of previously reported chronic exposures to poorly soluble particulates for each species and suggest that susceptibility of the rat, under pulmonary overload conditions, to the induction of lung tumors by these materials has underlying components of dosimetry and biological response.
Comments : Bermudez *et al.*, Toxicological Sciences 77, 347–357 (2004)
Previous study : Bermudez, E., Mangum, J. B., Asgharian, B., Wong, B. A., Reverdy, E. E., Janszen, D. B., Hext, P. M., Warheit, D. B., and Everitt, J. I.. Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol. Sci. (2002), 70, 86–97.

7. Repeated dose toxicity - chronic

   – Inhalation

P25

Country / Organisation: Germany
Responsible: Fraunhofer ITEM
Status:
Year of publication or finishing:
Method: Analysis of retained P 25 in lungs
Chronic toxicity / Carcinogenicity Chronic inhalation test
OECD 451/452, 2-year study / 30 months (lifetime)
Sample preparation method Aerosolization of the dry P 25 powder (MMAD: 0.8 µm - GSD: 1.8), concentration of test material: aerosol concentration of average 10 mg/m3 P-25 for exposure to rats/mice; reference material: carbon black, diesel exhaust
Test organism: rat /Wistar Crl: (WI)BR, mouse (strain NMRI
Results:
Comments :

Different types of nano TiO2

Country-Organisation : USA/NIOSH
Responsible:
Status:, published on website
Year of publication or finishing : 2005
Method: The National Institute for Occupational Safety and Health (NIOSH) has reviewed the relevant animal and human data for assessing the carcinogenicity of TiO2
Sample preparation method :
Test organism: rat and human (epidemiologic study)
Results: First, the tumorigenic effects of TiO2 exposure in rats appear not to be chemical specific or a direct action of the chemical substance itself. Rather, these effects appear to be a function of particle size and surface area acting through a secondary genotoxic mechanism associated with persistent inflammation. Second, current evidence indicates that occupational exposures to low concentrations of TiO2 produce a negligible risk of lung cancer in workers.
NIOSH recommends exposure limits of 1.5 mg/m3 for fine TiO2 and 0.1 mg/m3 for ultrafine TiO2, as time-weighted average concentrations (TWA) for up to 10 hr/day during a 40-hour work week. These recommendations represent levels that over a working lifetime should reduce risks of lung cancer to below 1 in 1000. These exposure limits were established using the international definitions of respirable dust [CEN 1993; ISO 1995] and the NIOSH Method 0600 for sampling airborne respirable particles [NIOSH 1998].
Comments : NIOSH CURRENT INTELLIGENCE BULLETIN: Evaluation of Health Hazard and Recommendations for Occupational Exposure to Titanium Dioxide, http://www.cdc.gov/niosh/review/public/tio2/pdfs/TIO2Draft.pdf

8. Local tolerance - Skin sensitisation

Material 12:

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: OECD 429 - Dermal Sensitization Test: Local Lymph Node Assay (LLNA) in Mice
Sample preparation method
Five groups of 5 female CBA/JHsd mice were dosed for 3 consecutive days with 0% (vehicle control), 5%, 25%, 50%, or 100% ultrafine TiO2 particle-types on both ears. N,N-dimethyl formamide was used as the diluting vehicle. One group of 5 female mice was dosed for 3 consecutive days with 25% hexylcinnamaldehyde (HCA) in 4:1 acetone: olive oil (AOO) as a positive control and one group of 5 female mice was dosed for 3 consecutive days with AOO as a positive control vehicle. On test day 5 of the assay, mice received 3H-Thymidine by tail vein injection and were sacrificed approximately 5 hours later. The cell proliferation in the draining auricular lymph nodes of the ears from the test substance groups was then evaluated and compared to the vehicle control group.
A stimulation index (SI) was derived for each experimental group by dividing the mean dpm (disintegrations per minute) of each experimental group by the mean dpm of the vehicle control group. The decision process in regard to a positive response includes an SI of greater than or equal to 3.0 together with consideration of dose response and, where appropriate, statistical significance. Significance was judged at $p < 0.05$ except for dpm data that were judged at $p < 0.01$. Lymph node dpm data were transformed to log to obtain normality or homogenous variances. When possible, an EC3 value for the stimulation index data was derived from linear interpolation of points on the dose-response curve immediately above and below the 3-fold threshold.
Test organism: mice
Results:
The objective of this study was to evaluate the potential of DLS (uf-C) TiO2 particles to produce a dermal sensitization response in mice using the local lymph node assay (LLNA).

No statistically significant differences in mean body weights and body weight gains compared to the vehicle control group were observed at any test concentration. No clinical signs of toxicity were observed in the study.
Statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at the 50% and 100% test concentrations. Stimulation indexes (SIs) of less than 3.0 were observed at all test concentrations of uf-C TiO2 particle-types. Therefore, the EC3 value (the estimated concentration required to induce a threshold positive response, i.e., SI=3) for the test substance under the conditions of this study was not calculable. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with ultrafine TiO2 particles.
Under the conditions of this study, DLS (uf-C) TiO2 particles did not produce a dermal sensitization response in mice.
Comments: Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

Material 18
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)

*Status:*published
*Year of publication or finishing :*2000
Method: A maximisation study was carried out in the guinea pig according to GLP and 84/449/EEC.
Sample preparation method : Twelve male and ten female animals of the Pirbright White strain were used. Two control groups consisted of 3 males and 3 females each.. The test group was composed of 6 males and 4 females. The active ingredient was T805. It was suspended in light paraffin oil at a concentration of 0.5%. This had been determined to be the maximum non-irritating concentration (when administered intradermally) in a preliminary experiment.
An area 6 X 8 cm in the scapular area was prepared by clipping. Three pairs of intradermal injections each of 0.1 ml, were made: (1) Freund's complete adjuvant (FCA) in physiological saline 1/1 (treatment and control animals); (2) active ingredient (T) or vehicle (C); (3) active ingredient + FCA 1/1 (T) or FCA + vehicle 1/1 (C).
On day 7, the scapular area was again clipped and a 10% aqueous solution of sodium lauryl sulphate was applied to the skin. On day 8, the area was exposed to a 30% solution of the test substance or the vehicle as appropriate and covered with an occlusive patch for 48 hours.
On day 21, areas of about 5 cm X 5 cm were clipped free of hair on both flanks of the animals. The left flanks of the test animals and the control group 1 animals were treated with 0.2 ml of the test substance at 5%, and the right flanks with 0.2 ml of the vehicle. These sites were covered occlusively for 24 hours. This procedure was repeated once. Observation was at 24 and 48 hours and scored on a Draize scale.
Test organism: guinea pig
Results: There were no reactions of any kind following the epidermal challenge. There was no evidence of sensitisation. There were no signs of systemic toxicity; body weight gain was as would be expected
Comments: From Opinion of the Scientific Committee on Cosmetic Products and non-food products intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. Degussa AG, US-IT No. 92-0036-DGT. 1992 : 'Titanium Dioxide T805 - Testing the cutaneous sensitising properties in the guinea pig (maximisation test)

Country-Organisation : European comission
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)
*Status:*published
*Year of publication or finishing :*2000
Method: A test for photo-sensitisation was carried out in 15 SPF guinea pigs, 8 males and 7 females.
Sample preparation method The test group comprised 5 males and 5 females; the control group 3 males and 2 females.. The test substance was T805®. The induction was in the nuchal region, in which 4 injections of Freund's complete adjuvant were made at the corners of an area which was then treated with 0.2 ml of a 30% suspension of the active ingredient in ethanol 5 times in two weeks. The negative control was vehicle only. UVA, 310-420 nm, was applied for about 50 minutes after each application, to give a dose of 10 J/cm2. Twelve days after the last induction procedure, the same areas were irradiated with the same dose of UVA. Observation was at 24 and 48 hours later.
Test organism: SPF guinea pigs
Results: No skin effects were observed, with or without irradiation. In the laboratory, there had been regular tests to the same protocol using musk ambrette as a positive control.
Comments: From Opinion of the Scientific Committee on Cosmetic Products and non-food products intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. Degussa AG, US-IT No. 92-0042-DGT. 1992. Acute dermal photoirritation study with Titanium Dioxide T805 in albino rats.

9. Local tolerance - Skin irritation:

Material 12:

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: publishe
Year of publication or finishing: 2007
Method: OECD 404 - Acute Dermal Irritation Study in Rabbits
The acute dermal irritation tests were conducted according to US EPA and OECD 404 guidelines (USEPA, 1998; OECD 2002).
Sample preparation method
Ultrafine TiO2 DLS (uf-C) particles were applied as a single 0.5g dermal dose to the shaved intact skin of 3 male New Zealand White rabbits. The test substance, moistened with 0.25 mL of deionized water, applied to a 6 cm2 area of skin. The application area was covered with a 2-ply gauze square which was held in place with non-irritating tape and covered with porous tape for a semi-occlusive dressing. The rabbits were exposed to the test substance for 4 hours after which the test substance was removed. Test sites were evaluated by Draize for signs of dermal irritation approximately 60 minutes, and 24, 48, and 72 hours after test substance removal. The rabbit that was initially treated was also examined immediately after test substance removal.
Test organism: rabbits
Results:
The rabbits exhibited no dermal irritation during the study. No clinical signs of toxicity were observed, and no body weight loss occurred. Under the conditions of this study, DLS (uf-C) TiO2 particles were not considered to be skin irritants.
Comments: Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

Material 18

Country-Organisation : European comission
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)
*Status:*published
*Year of publication or finishing :*2000
Method: The commercial preparation T805 was tested according to GLP and 92/32/EEC in 3 Russian white rabbits.
Sample preparation method :Two areas of the skin of the back were clipped the day before the experiment. One area was for the test, the other served as a control. A dose of 0.5 gram of the test substance was moistened with 0.64 ml of paraffin oil, and applied to the skin on the left side of the vertebral column and covered with an occlusive dressing for 4 hours. The site on the opposite side was treated identically except that no active ingredient was applied. Reading was at 24, 48 and 72 hours.
Test organism: Russian white rabbits
Results: There was slight erythema (grade 1) at 24 hours in 2 animals at 24 hours, absent at 48 hours. Slight oedema (grade 1 at 24 hours) was noted in one animal, absent at 48 hours. There were no systemic toxic effects. The primary irritation index was 0.3/8, so the material was judged to be non-irritant.
Comments: From Opinion of the Scientific Committee on Cosmetic Products and non-food products

intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. Degussa AG, US-IT-No. 93.0058-DGT. 1993. Titanium Dioxide T805, Acute Toxicity, Testing the primary irritation/corrosion after single application to the skin of the rabbit (patch test).

Country-Organisation : European comission
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)
*Status:*published
*Year of publication or finishing :*2000
Method: acute dermal photoirritation was carried out using the preparation T805.
Sample preparation method :The test animals were 3 female NZW rabbits. Single applications of the material in ethanol in concentrations of 3%, 10% and 30% were made to the skin of both flanks for 100 minutes, without occlusion; one flank was irradiated with UVA, 310 to 420 nm, for 50 minutes, to administer 10 J/cm2. The areas were inspected at 30 minutes, and 24, 48 and 72 hours. There were no signs of photo-irritation.
Test organism: NZW rabbits
Results: There were no signs of photo-irritation.
Comments: From Opinion of the Scientific Committee on Cosmetic Products and non-food products intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. Degussa AG, US-IT No. 92-0042-DGT. 1992. Acute dermal photoirritation study with Titanium Dioxide T805 in albino rats.

10. Local tolerance - Eye irritation

Material 12:

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: OECD 405 - Acute Ocular Irritation Study in Rabbits
The acute eye irritation tests were conducted according to US EPA and OECD 405 guidelines (USEPA, 1998; OECD, 2002).
DLS (uf-C) TiO2 particles were evaluated for acute eye irritation potential in 3 young adult New Zealand White rabbits. The study was conducted after confirming that the compound was not a severe irritant or corrosive to the skin.
Sample preparation method
Approximately 57 mg of test substance was administered to 1 eye of each animal. The eyes remained unwashed following treatment. One rabbit was initially treated. Since no severe irritation or corrosion was observed, 2 additional rabbits were treated to complete the test. The conjunctiva, iris, and cornea of each treated eye were evaluated and scored according to a numerical scale approximately 1, 24, 48, and 72 hours following administration of the test substance.
Test organism: rabbits
Results:
The test substance produced conjunctival redness (score 1 or 2) in the treated eye of all three rabbits. Fluorescein stain examinations did not reveal any corneal injury. The treated eyes of the rabbits were normal by 24 or 48 hours after instillation of the test substance. No clinical signs were observed, and no body weight loss occurred.
Based on this study, DLS (uf-C) ultrafine TiO2 particles produced conjunctival redness in the treated rabbit eye which was reversible.

Comments: Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

11. Reproductive toxicity, Developmental toxicity (if available)

Material 14,15,16

Country / Organisation: Denmark, NRCWE
Responsible:
Status: in progress
Year of publication or finishing:
Method: adapted OECD guidelines and other.s The mentioned repro and behavioral study is only completed on one of the TiO2 samples at this point.
Sample preparation method
Test organism:
Results:
Comments ::

12. Genotoxicity

a. In vitro genotoxicity

P 25:
Remark :Theogaraj E. et al., Mutation Research 634 (2007) 205-219, Comparable to OECD Guideline 473

Country-Organisation : France/INRS
Responsible: Yves Guichard
Status: in progress
*Year of publication or finishing :*Expected completion in early 2010
Method: In vitro mammalian cell micronucleus test: OECD, draft guideline 487 - Morphological transformation in SHE: method described in Leboeuf *et al.,* 1996, Mutation, Res., 356:85-127 - Cell growth inhibition: method described in Elias and al., 1995, Cancer Detect Prev., 19:405-414 - Apoptosis: Annexin-V FITC detection
Sample preparation method :
Particle powders are suspended in medium and homogenised by sonication
0,5 µg/cm^2 to 200 µg/cm^2
Ultra-pure water, Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal bovine serum and antibiotics (Penicillin,Steptomycin)
Test organism: Syrian hamster embryo (SHE) cells
Results:
Comments: Material used :Anatase, Sigma, cat 637254, batch 07324KD, dots, 6 ± 14 nm - Rutile, Sigma, cat 637262, batch 07819DD, rods, 62 ± 24 nm x 10 ± 2 nm - Anatase/Rutile (80%/20%), Degussa, P25, batch 23.8595.0000.01, dots, 25 ± 6 nm

Material 2

Country-Organisation : Germany/BASF SE
Responsible:
Status: experimental result
Year of publication or finishing :Method: in vitro mammalian cell micronucleus test ,GLP guideline study, OECD 487, draft
Sample preparation method :
Test organism: Chinese hamster lung fibroblasts (V79)
Results: all strains/cell types tested, Genotoxicity : negative
Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)

Material 2,4

Country-Organisation : Germany/BASF SE
Responsible:
Status: exerimental results
*Year of publication or finishing :*2009
Method: bacterial reverse mutation assay (e.g. Ames test) according to OECD Guideline 471 (Bacterial Reverse Mutation Assay)
Sample preparation method :
Test concentrations :5 doses with a maximum of 5000 µg/plate
STANDARD PLATE TEST
The experimental procedure of the standard plate test (plate incorporation method) is based on the method of Ames et al. (Mut. Res. 31:347-364, 1975; Mut. Res. 113:173-215, 1983).
PREINCUBATION TEST
The experimental procedure is based on the method described by Yahagi et al. (Mut. Res. 48:121-130, 1977) and Matsushima et al. (In: Norpoth, K.H. and R.C. Garner, Short-Term Test Systems for Detecting Carcinogens. Springer Verlag Berlin, Heidelberg, New York (1980)).
TOXICITY
The toxicity is detected by a decrease in the number of revertants and/or by a clearing or diminution of the background lawn (= reduced his- background growth).

EVALUATION CRITERIA
The test substance is considered positive in this assay if a dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain, is observed.
A test substance is generally considered non-mutagenic in this test if the number of revertants for all tester strains are within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.
Test organism:: S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 102
Results: all strains/cell types tested, Genotoxicity : negative
Comments : Company data from an internal report 13463-67-7_master_titanium dioxide (IUC4 DSN 553)

Material 12:

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: Bacterial Reverse Mutation (Ames) Test using the plate incorporation method.
DLS (uf-C) ultrafine TiO2 particles were evaluated for mutagenicity in the Bacterial Reverse Mutation (Ames)
Test using the plate incorporation method. Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were tested in the absence and presence of an exogenous metabolic activation system (Aroclor-induced rat liver S9). Sterile water was chosen as the dosing vehicle based on the solubility of the test substance and compatibility with the target cells. The test substance formed a homogeneous suspension at 50mg/mL, the highest concentration that was tested in the study. A plating aliquot of 100 µL was used. This dose was achieved using a concentration of 50 mg/mL and a 100 µL plating aliquot. The dose levels in the study were 100, 333, 1000, 3333, and 5000 µg per plate. Appropriate positive controls were included in the study. The study was conducted according to the US EPA and OECD 471 testing guidelines (USEPA, 1998; OECD, 1998).
Milli-Q water was chosen as the dosing vehicle for the Ames test based on compatibility with the target cells. The test substance formed a homogeneous suspension at 50 mg/mL, the highest concentration that was tested in the study. A plating aliquot of 100 µg/mL was used. The dose levels in the study were 100, 333, 1000, 3333, and 5000 µg per plate.
Test organism: Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA
Results:
No positive mutagenic responses or compound-related toxicity were observed at any dose level with any tested strain (Salmonella typhimurium tester strains TA98, TA1000, TA 1535, TA 1537, or Escherichia coli strain WP2uvrA) when tested either with or without an S9 metabolic activation system (Arocolor-induced rat liver S9). Compound precipitate was observed at the top three or four dose levels.
DLS (Uf-C) ultrafine TiO2 particles showed no evidence of mutagenicity in this study.
Comments : Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

Country / Organization: BIAC
Responsible: DuPont Haskell Laboratory; D.B. Warheit et al.
Status: published
Year of publication or finishing: 2007
Method: OECD 473 - In Vitro Mammalian Chromosome Aberration Test in Chinese Hamster Ovary Cells
Test organism: Chinese Hamster Ovary Cells
Results:
DLS (uf-C) ultrafine TiO2 particles were tested for their ability to induce structural chromosome aberrations in Chinese hamster ovary (CHO) cells in the absence and presence of an exogenous metabolic activation system (Aroclor-induced rat liver S9).
DLS (uf-C) ultrafine TiO2 particles did not induce structural or numerical chromosome aberrations in this study.
Comments: Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, and Sayes CM. Development

of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99- 110, 2007.

Material 18
Country-Organisation : European comission
Responsible: SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products)
*Status:*published
Year of publication or finishing: 2000
Method: different methods bacterial assay ,Chromosome aberration, Neutral Red Uptake Photo-toxicity test
Sample preparation method :.
Test organism: Salmonella typhimurium, Escherichia coli, Chinese Hamster Ovary (CHO) cells
Results: The results of these tests were : negative.
Comments: From Opinion of the Scientific Committee on Cosmetic Products and non-food products intended for consumers concerning Titanium Dioxide. Colipa n° S75. Adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000.

- Degussa AG, US-IT No. 94-0181-FGM (1994), Titanium Dioxide T 805: Reverse mutation assay (Ames test) using Salmonella typhimurium and Escherichia coli.
- Degussa AG, US-IT No. 98-0092-DGM (1998), Titanium dioxide T 805: Induction of chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells.
- Degussa AG, US-IT No. 98-0014-DGM (1998), Titanium dioxide T 805: Reverse mutation in three histidine-requiring strains of Salmonella typhimurium and a tryptophan-requiring strain of Escherichia coli, in the presence of ultra violet light.
- Degussa AG, US-IT No. 98-0073-DGM (1999), Titanium dioxide T 805: Induction of chromosome aberrations in cultured Chinese hamster ovary (CHO) cells in the presence of ultra violet light.
- Beiersdorf AG, Test report (7436/PZK.111), No 1110.0409-32-28 (1999), Testing of Titandiox T805 (Degussa-Hüls AG, 05 10067) versus Titandiox P25 (Degussa-Hüls AG P1S-3087) with the 3T3 Neutral Red Uptake Phototoxicity Test.

b. In-vivo genotoxicity,

It should be addressed in the sponsorship Programme, Phase 2

P 25:
Germany, BASF: Comet assay (*in vivo*, rat)

P25, and other Materials

Country-Organisation : France/ CEA Saclay
Responsible: Marie Carriere, Nathalie Herlin
Status: in progress
Year of publication or finishing :
Method: genotoxicity: comet assay, gamma-H2AX immunostaining, micronucleus assay toxicogenomics: microarray
Sample preparation method cell culture media - hoagland medium
Test organism: A549, HepG2, WIF-B9, Can10, NRK-52E, LLC-PK1, MDCK
Results:
Comments : TiO2 purchased from sigma, degussa and synthesized by laser pyrolysis in DSM/IRAMIS/SPAM laboratory (CEA Saclay)

c. In vivo germ cell mutagenicity

France: (planned project)

Others In vitro Tocixity

P 25, Material 1to 6

Country-Organization: USA, US EPA
Respsonsible: Kevin Dreher, US EPA
*Status :*initiated, in progress
Year of publication or finishing :
Methods, Tests : US EPA is currently employing an integrated testing stratey consisting of alternative test methods (cellular/in vitro and non-cellular test methods) and *in vivo* or mammalian toxicity testing to assess the health effects of nanoTiO2.
All TiO2 nanomaterials have undergone independent physical, chemical and biological characterizations prior their use in this integrated testing strategy.
***In vitro* toxicity** testing employing alternative testing employing is underway using a variety of cell types reflecting different routes of exposure (dermal, inhalation, ingestion) as well as to assess the health effects that may arise due to the ability of nanomaterials to translocate from their initial site of deposition to other organs of the body. Nanomaterials *in vitro* toxicity testing will assess mutagenic, intestinal, pulmonary, dermal, immunological, neurological, reproductive, cardiovascular, ocular, and developmental toxicities using cellular models reflective of these toxicities.
In Vitro testing endpoints: cellular toxicity; apoptosis; cytokine production; reactive oxidant stress; and measurements associated with alteration in permeability and specific cellular functions depending on target cell type. Alternative test methods using non-cellular assays are being employed to examinine: particle dispersion/aggregation; zeta charge; reactivity and capability to generate reactive oxygen specices; photocatalytic potential; capability to modify proteins and deplete antioxidants and second messengere. Non-cellular interactions and surface properties of TiO2 nanomaterials may play a key role in regulating their cellular uptake and toxicity

***In vivo or* mammalian toxicity** testing will be conducted in mice and/or rats to assess pulmonary toxicity, immunotoxicity and cardiovascular toxicity following intratreacheal instillations and inhalation to TiO2 particles. In vivo toxicity endpoints will include but will be limited to: inflammation, cell proliferation, fibrosis, necrosis, apoptosis, coagulation, cellular activation, and biomarkers of oxidative stress. Alterations in cardiac and vascular physiological responses will be assessed following intratreacheal instillations and inhalation to TiO2 particles.

Test organism :
***In vitro** testing* will be conducted on Cell lines or models employed in *in vitro* toxicity test screening include: pulmonary and immune toxicity (human airway epithelial cells; alveolar macrophages; alveolar epithelial cells derived from primary cultures and the BEAS2B cell line); cardiac toxicity (rat cardiomyocytes); vascular toxicity (human endothelial cells); neurotoxicity (rodent glial cells; neuronal cells); reproductive toxicity (whole rodent embryo culture); occular toxicity (HLE B-3; APRE 19); carcinogenicity (BEAS2B; Caco2; NCM460).
In vivo or mammalian toxicity testing will be conducted in mice and/or rats
Sample Preparation:
In vitro testing - Particles examined by alternative test methods are resuspended and sonicated in appropriate tissue culture media for and saline or physiological phosphate buffered saline for non-

cellular assays.
***In vivo* or mammalian toxicity** testing – pulmonary exposures using intratracheal instillation samples are resuspended and sonicated in physiological phosphate buffered saline. To minimize oral ingestion/exposures of TiO2 particles acute and subacute inhalation exposures of rodents are performed in nose only chambers to aerosolized TiO2 while monitoring particle mass concentration in $\mu g/m^3$, size mode distribution, and particle number concentration.
Results: None to report.
Comments: It is anticipated that *in vivo* or mammalian toxicity testing of specific TiO2 particles will be guided by results generated from alternative toxicity testing studies. *In vitro* and non-cellular test methods will need to be validated in parallel mammalian toxicity testing studies.

P 25and other Materials

Country-Organisation : France/ CEA Saclay
Responsible: Marie Carriere, Nathalie Herlin
Status: in progress
Year of publication or finishing :
Method: cytotoxicity: MTT, LDH, WST-1 oxidative stress: H2DCF-DA, SOD, catalase, GRED, GSH activity
TiO2 purchased from sigma, degussa and synthesized by laser pyrolysis in DSM/IRAMIS/SPAM laboratory (CEA Saclay)
*Sample preparation method :*cell culture media - hoagland medium
Test organism: A549, HepG2, WIF-B9, Can10, NRK-52E, LLC-PK1, MDCK
Results:
Comments :

Country-Organisation : Germany
Responsible: NanoCare
Status: in progress
Year of publication or finishing :
Method: different material (Size, surface charge) and different in vitro methods with various mamalian cell types, phathophysiological investigation
Sample preparation method
Test organism: various mamalian cell types,
Results:
Comments :

Country-Organisation : Germany
Responsible: Fraunhofer IKTS
Status: will be finished
Year of publication or finishing :
Method: different material (Size, surface charge) and different in vitro methods with various mamalian cell types, phathophysiological investigation
Sample preparation method : different media (NaCl solution, PBS, HBSS, DMEM) with and without BSA or FBS;
Test organism: Human cell lines: lung, skin, bowel prime cells, neural cells, glia cells
Results:
Comments : particle morphology (TEM), FESEM, Particle size distribution, dustiness, apparent

density, BET surface area ASAP 2010 (N2-Adsorption), Zeta Potential (Zeta Sizer MALVERN), determination of agglomeration, aggregation during the test

Material 7:

Country-Organisation : Germany
Responsible: Fraunhofer ITEM (2.2)
Status: in progress
Year of publication or finishing :
Method:. Influence of TiO2 particles on pulmonary surfactant (measuring of surface tension in a pulsating bubble surfactometer) before and after 'surface area cycling (in vitro simulation of breathing) and influence on surfactant ultrastructure (TEM pictures). no guideline applicable
Sample preparation method : Preparation of particle suspension; vortexing and ultrasonic before use; Concentration of test substance: 0 µg/ml - 500 µg/ml;
Test organism:
Results:
Comments : reference material: Microsized TiO2 particles;alpha-quartz; nanosized and microsized polystyrene particles with or without surface coating

Material 9 and others

Country-Organisation : France/Inserm
Responsible: Sophie Lanone
Status:, in progress
Year of publication or finishing :2008
Method: In vitro toxicity on human pulmonary fibroblasts, extracellular matrix components, oxidative stress, inflammation. TiO2 suspension in culture medium
Test method : MTT, Neutral Red, DNA quantification. Vortexing and ultrasonic processing before exposureto cells
Sample preparation method : Cell culture medium
Test organism: MRC5 cells
Results:
*Comments:*in progress will end late 2011. Material tested in addtion with n° 8 : Pure anatase 10 and 15 nm, Mix anatase/rutile 25-75 nm, round 30-40 nm or needle-like rutile 10*40 nm which is coated with 5% SiO2 fromNanoamor (except for anatase 15 nm and mix anatase/rutile which are from Sigma Aldrich).

Material 19

Country-Organisation : USA/Dupont
Responsible: Sayes, C.M. and Warheit, D.B.
Status: published
Year of publication or finishing : 2008
Method: The aim of this study was to compare the cytotoxicity endpoints in two different lung epithelial cell lines following in vitro exposures to well characterised nano (or ultrafine) and fine-sized TiO2 particles and, subsequently, compare them to previously reported in vivo lung toxicity data. Cells in culture (human lung epithelial (A549) or rat lung epithelial cells (L2)) were incubated with doses of 0.8, 8.0, or 16.0 µg/cm2 of the following rutile TiO2 particle-types: ultrafine-sized

TiO2 particles with an alumina surface coating; ultrafine-sized TiO2 particles with an alumina and amorphous silica surface coating; fine-sized TiO2 particles with an alumina surface coating; or fine-sized TiO2 particles with an alumina and amorphous silica surface coating. Following incubation, the particle-exposed cells were evaluated for cytotoxicity endpoints in the culture media, including lactate dehydrogenase (LDH) release, 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) activity, and changes in micro-total protein at post-incubation time points of 1, 4, 24, and 48 h.
The protocl of in vivo lung toxicity studies in rats is already described*
Sample preparation method : F-12K medium (Kaighn's modification of Ham's F-12 medium) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. All experiments were conducted at a cellular density of 250,000 cell/cm2.
Test organism: immortalised human lung epithelial cells (A549); immortalised rat lung epithelial cells (L2); rats
Results: Results showed that cytotoxicity responses of human lung epithelial cells were similar, but not identical, to responses of rat lung epithelial cells. Generally, the ultrafine TiO2 particles increased the levels of LDH in the culture media of cells more often than the fine TiO2 particle-types. These in vitro findings were not consistent with results measured in previous in vivo studies in the lungs of rats using the same particle-types. In the in vivo system, neither the fine, nor ultrafine, TiO2 particles produced sustained cytotoxic effects in the lung fluids of rats. Further, results indicate that the 48 h time point in the in vitro time course study does not accurately reflect the cytotoxicological response of these nanoparticle-types.
Comments In vitro techniques will need to be further developed and validated. Further, other particle-types will need to be tested in order to provide useful screening data for predicting in vivo pulmonary toxicity for a variety of nanoparticles. (Warheit, D.B. (2008) 'An in vitro investigation of the differential cytotoxic responses of human and rat lung epithelial cell lines using TiO2 nanoparticles', Int. J. Nanotechnol., Vol. 5, No. 1, pp.15–29.

* Warheit, D.B., Webb, T.R. and Reed, K.L. (2006) 'Pulmonary toxicity screening studies in male rats with TiO2 particulates substantially encapsulated with pyrogenically deposited, amorphous silica', Particle and Fibre Toxicology, Vol. 3, No. 3, pp.1743;
Warheit, D.B., Carakostas, M.C., Hartsky, M.A. and Hansen, J.F. (1991) 'Development of a short-term inhalation bioassay to assess pulmonary toxicity of inhaled particles: comparisons of pulmonary responses to carbonyl iron and silica', Toxicol. Appl. Pharmacol., Vol. 107, pp.350–368.

Different Materials

Country-Organisation : France/Ecole des Mines de Paris/CEA
Responsible: Jesus Angulo
Status: in progress,
Year of publication or finishing : 2008
Method: innovative Cell on chip technology and relate it to the physico-chemical NP characteristics.
Sample preparation method : DropChip prototype: formation of 100nl drops on 400 spots using a piezoelectric nozzle. By automated image capture by the microscope, each spot is analysed using several fluorescent wavelengths (the super-imposition of the blue Hoechst signal, for nucleus detection, the red Ethidium signal for the qualification of cell death, and the green SYTO 10 signal, specific of living cells)
Test organism:
Results: PARTOX project's goal is to assess the human toxicology of nanoparticles (commercial and specially tailored NP), using innovative Cell on chip technology and relate it to the physico-chemical NP characteristics
Comments: Rahman et al Environmental Health Perspectives • VOLUME 110 | NUMBER 8 |

August 2002)
Materials used : Alfa Aesar (ref : AA36199, AA39953, AA42681,AA44517,AA44689,AA44690), and Sigma Aldrich (SA637262).

P 25 and Several types of nano TiO2

US EPA: health effects associated with size, surface area, and crystalline structure (Degussa P-25 TiO2, 86 % anatase/14% rutile, ~21 nm; 10 nm anatase; 30-40nm rutile; 32nm anatase; 200nm anatase; 250nm rutile)
For in vitro toxicity testing additional nanoparticle characterization is being conducted in order to characterize nanoparticle in liquid suspensions that will be used in dosing or exposure.
Cell- Lines: In vitro toxicity test screening: pulmonary tox. (human airway epithelial cells; macrophages; alveolar epithelial cells); cardiac tox. (rat cardiomyocytes); vascular tox. (human endothelial cells); neurotox. (rodent glial cells; neuronal cells); reproductive tox. (whole rodent embryo culture); occular toxicity (HLE B-3; APRE 19); carcinogenicity (BEAS2B; Caco2; NCM460). Future, in vivo mammalian toxicity testing will be conducted in mice and/or rats and guided by in vitro tox. test data.
These characterization includes DLS; SEM; TEM; cryoTEM for size determination in liquid/suspension form; zeta potential. In vivo animal tox. testing will involve similar characterizations for dermal and oral exposures while inhalation exposures will include mass, aerodynamic and particle size distribution characterization of the aerosol. Sample Preparation: Methods are under development to generate and characterize nano TiO2 liquid suspensions. Methods are being developed to generate dry aerosols of nano TiO2.Under development, focusing on stirring first, avoiding solvents or dispersants, sonicating as potential treatment. Surface area, purity, crystal form were characterized.

The various nanoTiO2 are currently undergoing an in vitro toxicity srceening for ranking in the following in vitro toxicity models: pulmonary toxicity; cardiac and vascular toxicity; neurotoxicity; mutagenesis/carcinogenesis toxicity; occular toxicity; and reproductive toxicity. Future toxicity testing will involve in vivo or animal toxicity testing based on data generated from these in vitro test that will rank nanoTiO2 particles for in vivo mammalian tox. testing and assist in their experimental design with respects to dose and endpoints to exmaine.
For in vitro toxicity testing additional nanoparticle characterization is being conducted in order to characterize nanoparticle in liquid suspensions that will be used in dosing or exposure. These characterization includes DLS; SEM; TEM; cryoTEM for size determination in liquid/suspension form; zeta potential. In vivo animal tox. testing will involve similar characterizations for dermal and oral exposures while inhalation exposures will include mass, aerodynamic size, particle size, characterizations of the aerosol. In vitro mammalian and non-cellular assays are currently being conducted. In vivo mammalian toxicity testing is planned.

13. Experience with human exposure (if available)

Material safety (if available)

Flammability
Material 12:
Non-combustible

Explosivity
Material 12:
Non-explosive refractory oxide

In progress in France, informations will be provided later

Incompatibility
Material 12:
No known incompatibility

ANNEX 1: SPREADSHEET

Titanium Dioxide

Country/Organization

Researcher contact information
- name, address, phone, fax, email

Description of tested Material
- size, shape, crystal form, batch number ...

- coating yes/no
  description of the coating (material)

Material Source
- (Company name, contact information, lab-produced research quantity, etc.)

Test (Endpoints from OECD list)

- P/Chem properties - Endpoint

- Environmental fate - Endpoint
(e.g. Biodegradation / DOC)

- Ecological toxicity - Endpoint
(e.g. acute toxicity to fisch)

- Mammalian toxicity - Endpoint
(e.g. in vivo, inhalation or dermal toxicity test)

- additional endpoints/observations during the test
e.g. Histopathology
cell proliferaion/appoptosis

- Other Endpoints
(Endpoints which are not included in the OECD list)

Methods

Test Method
- used or being considered for use (e.g., OECD guideline number)
or short description of other test method

Adaptation of Test method
- if you use a standard method: the kind of adaptation

Sample Preparation Method

Concentration of Test substance
(for tests in progress or completed)

Reference Material

List of known material characteristics

- Characterized by
(manufacturer, researcher, independent lab, etc.)

- As-produced material
- In exposure media

test media and test organism

Test media
- (e.g. Hank's buffer, freshwater, marine/saline water, sediment, terrestrial soils, sludge, etc.)
Tests with standard methods: all modifications of the test media

Agglomeration/Aggregation during the test
- measuring: yes/no

Organisms or cell-line tested
(or to-be tested)

administrative data and others

Other Information

Status of Testing
- (completed, in progress, planned. If in progress or planned: date of the expected completion)

Date of data entry

ANNEX 2: DDP DRAFT

TiO2 DDP Draft

Phys-chem Properties

DDP Responsibility: Germany and France

Endpoint	***P25***	***Addressed by***
Agglomeration/aggregation	X	Austria, Canada, Germany
Water solubility	X	Germany
Crystalline Phase	X	Germany
Dustiness	X	Germany, Denmark
Average crystallite size	X	Germany
Representative TEM pict	X	Sponsors/co-sponsors
Particle size distribution	X	Sponsors/co-sponsors
Specific surface area	X	Sponsors/co-sponsors
Zeta potential	X	Sponsors/co-sponsors
Surface chemistry	X	Sponsors/co-sponsors
Photocatalytic activity	X	US
Pour density	X	Germany
Porosity	X	Germany
Octanol-water partition coe	-	Only relevant for coated products
Redox potential	-	Not relevant
Radical formation potential	gap	Clarification neccessary

Environmental Fate

DDP Responsibility: Germany

Endpoint	*P25*	*Addressed by*	*Other material*
Dispersion stab / water	X	Germany, Austria	
Biotic degradability	-	Not relevant	
Ready biodegradability	-	Not relevant	
Simul testing /surf water	X	Germany, Austria	5
Soil simulation testing	X	Canada	
Sediment simulation testing	-	Not relevant	
Sewage treatment sim testing	-	Not relevant	
Identification of degr products	-	Not relevant	
Further testing of degradat prod	-	Not relevant	
Abiotic degrationd and fate	-	Not relevant	
Hydrolysis	-	Not relevant	
Adsorption/Desorption	gap		
Bioaccumulation	gap	Germany, France (planned)	

Environmental Toxicology

DDP Responsibility: Germany

Endpoint	***P25***	***Addressed by***	***Other material***
Algae	X	Germany, Spain, Denmark, BIAC	5, 8,9,10,11,12, other
Daphnia	X	Germany, Spain, Canada, Denmark, BIAC	3, 4,5,12
Fish	X	Canada, Spain, US, BIAC	10, 12
Effects on Sediment Species	gap	Germany (planned)	
Effects on Soil Species	gap	Canada; Other materials, not for P 25	8,11
Effects on terr Species	gap	Germany (planned)	
Effects on microorg	gap	Germany, France (planned)	
Plankton communities	X	Spain	10

Mammalian Toxicology

DDP responsibility: France

Endpoint	*P25*	*Addressed by*	*Other material*
Pharmacokinet -ADME	X	Germany, Switzerland, US, France	other
Acute Tox - Inhalation	X	France, Germany, BIAC, Denmark	1,2,6,12,14,15,16
Acute Tox - Oral route	gap	BIAC other material	12
Acute Tox – Dermal	gap	Germany, BIAC other material	2, 3, 12
Skin sensitation	X	ICCP (CEFIC), BIAC other material	6, 12
Eye irritation	X	ICCP (CEFIC), BIAC other material	6, 12
Repeated dose toxicity	X	Germany, CEFIC	14,15,16
Chronic toxicity	X	Germany	
Reproductive toxicity	gap	Denmark, also planned for P25	14,15,16
Developmental toxicity	gap	Denmark, also planned for P25	14,15,16
Genetic tox – in vitro	X	Germany, Denmark	2,4,14,15,16 other
In vivo – Germ cell tox Comet Assay	X	France (planned), BIAC	
Other relevant data* Neurotoxicity Histopathology In vitro tests – SG 7	X	Germany, US, China	7