

TEXTE

69/2016

# Summary of the results of the Sponsorship Programme for nanoscale titanium dioxide of the OECD Working Party on Manufactured Nanomaterials



# **Summary of the results of the Sponsorship Programme for nanoscale titanium dioxide of the OECD Working Party on Manufactured Nanomaterials**

by

Dr. Doris Völker and Dr. Kathrin Schwirn  
German Environment Agency  
Wörlitzer Platz 1  
06844 Dessau-Roßlau  
Germany

December 2015

On behalf of the German Environment Agency

# Imprint

**Publisher:**

Umweltbundesamt  
Wörlitzer Platz 1  
06844 Dessau-Roßlau  
Tel: +49 340-2103-0  
Fax: +49 340-2103-2285  
info@umweltbundesamt.de  
Internet: www.umweltbundesamt.de

 /umweltbundesamt.de

 /umweltbundesamt

**Study performed by:**

German Environment Agency  
Wörlitzer Platz 1  
06844 Dessau-Roßlau  
Germany

**Study completed in:**

December 2015

**Publication as pdf:**

<http://www.umweltbundesamt.de/publikationen>

ISSN 1862-4804

Dessau-Roßlau, October 2016

## Preface

This report summarizes the results of the Sponsorship Programme on ecotoxicology and environmental behavior of nanoscale titanium dioxide of the OECD Working Party on Manufactured Nanomaterials (WPMN). The data mentioned in this report were extracted from the dossier of the WPMN on nanoscale titanium dioxide. This dossier is published at: <http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm>

## Table of Contents

List of Tables .....	4
1 The Sponsorship Programme: motivation and objectives, structure and procedure .....	5
2 Scientific results of the OECD Sponsorship Programme on nanoscale titanium dioxide with regard to environmental behaviour and ecotoxicology .....	7
2.1 Scientific findings on the environmental behaviour of nTiO <sub>2</sub> nanomaterials .....	8
2.2 Scientific findings on the ecotoxicity of nTiO <sub>2</sub> nanomaterials .....	8
2.3 Summary and Outlook .....	10
3 Need for adjustment of the methods used to record ecotoxicology and environmental behaviour in the OECD Sponsorship Programme for nanoscale titanium dioxide: Perspective of the German Environment Agency .....	10
4 Research needs in the field of ecotoxicology and environmental behaviour identified on the basis of the results of the OECD Sponsorship Programme.....	13

## List of Tables

Table 1:	Übersicht der im SSP zu nTiO <sub>2</sub> betrachteten, repräsentativen Materialien .....	6
Table 2:	Übersicht zum Überarbeitungs- und Ergänzungsbedarf der im Sponsorship Programm angewandten Testrichtlinien der OECD für die Bereiche Umweltverhalten und Ökotoxikologie .....	12

## 1 The Sponsorship Programme: motivation and objectives, structure and procedure

The Sponsorship Programme is the central testing programme of the OECD Working Party on Manufactured Nanomaterials (WPMN) launched in 2007. It was called into being to create the conditions for a coordinated approach between the member countries with regard to the safety of manufactured nanomaterials. The WPMN brings together 30 member countries of the OECD and the EU Commission. Some non-member countries are also represented, as are organisations from industry and from standardisation and environmental associations. Germany is significantly involved in the activities being carried out under the auspices of the WPMN. The Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB) is responsible for chairing the German delegation. The party responsible for coordinating the German contribution to the OECD Sponsorship Programme was the German Environment Agency.

The idea behind the OECD Sponsorship Programme was to collate data on selected and precisely described manufactured nanomaterials. The intention was thereby to obtain information on their physicochemical properties, behaviour and effects on humans and the environment, and to demonstrate the relationships between them. This information was also intended to reveal differences between the inherent properties of specifically selected variants of the various nanomaterials. These variants differ in respect of their chemical composition, size, shape or surface finish. A further priority of the programme was to study the applicability to nanomaterials of those standardised test guidelines of the OECD which are used to study the behaviour of chemicals and their effects on humans and the environment.

In the first step, 14 chemical substances were selected, various nanoscale variants of which were to be investigated in a comparative study in the test programme<sup>1</sup>. This list was later amended to a total of 11. These are:

- Fullerenes (C<sub>60</sub>)
- Single-walled carbon nanotubes (SWCNT)
- Multi-walled carbon nanotubes (MWCNT)
- Nanoscale silver (nAg)
- Nanoscale titanium dioxide (nTiO<sub>2</sub>)
- Nanoscale cerium dioxide (nCeO<sub>2</sub>)
- Nanoscale zinc oxide NM (nZnO)
- Nanoscale silicon dioxide NM (nSiO<sub>2</sub>)
- Dendrimers
- Nanoclays
- Nanoscale gold (nAu)

---

<sup>1</sup> List of manufactured nanomaterials and list of endpoints for phase one of the sponsorship programme for the testing of manufactured nanomaterials [ENV/JM/MONO (2010) 46]: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2010\)46&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2010)46&doclanguage=en)



The criteria that led to the selection of these chemicals and their respective nanoscale variants included their market relevance or maturity, production quantities, qualitative and quantitative availability for the testing programme and the amount of any previously available information.

The participants in the Sponsorship Programme agreed among themselves on the responsibility they were to assume for the gathering of information on the individual nanomaterials. According to this agreement, the “Lead Sponsors” were responsible for the collation of information on all variants of one particular chemical substance. This process was supported by “Co-Sponsors” who assumed partial responsibility for specific actions, taking charge, for instance, of the collation of data for a sub-area or a particular variant. “Contributors” assisted in the data collation by feeding available information from existing national research projects into the programme.

In this process Germany joined forces with France as joint “Lead Sponsors” for nTiO<sub>2</sub>, acted as a “Co-Sponsor” with partial responsibility for nAg and contributed in the role of “Contributor” to the collation of data on nCeO<sub>2</sub>, nZnO, MWCNT, SWCNT and nAu. Alongside Germany and France as “Lead Sponsors”, Austria, Canada, South Korea, Spain, the US, the European Commission and BIAC<sup>2</sup> offered their support in the collation of data on nTiO<sub>2</sub>. Contributions came from Denmark, Japan and the United Kingdom as “Contributors”. Over 30 research institutions from these countries contributed with their results to the completion of the dataset.

The participants in the programme agreed on 59 “endpoints”<sup>3</sup> to be established for the variants of the selected nanomaterials. These endpoints included information on 1) the identity of the nanomaterial, 2) physicochemical characterisation, 3) environmental behaviour, 4) ecotoxicology, 5) toxicology, and 6) safety-related characteristics. This information was to be collated through experiments or using research from existing projects and publications. A guidance manual explaining the principles and methods of the Sponsorship Programme was drafted to ensure the consistency and, consequently, comparability of the work to be carried out by the various sponsors<sup>4</sup>.

To support the testing programme the WPMN published a guidance manual on the preparation and execution of testing<sup>5</sup>.

The following materials were selected for the testing programme for nTiO<sub>2</sub>:

Table 1: Overview of representative materials considered in the Sponsorship Programme on nTiO<sub>2</sub>

TiO <sub>2</sub> NM acronym	Trade name (manufacturer)	properties (primary particle size in nanometres [nm], crystal structure, where applicable surface modification)
NM 105*	Aeroxide P25 (Evonik)	21nm, anatase/rutile
NM 101	Hombikat UV 100 (Sachtleben)	10nm, anatase
NM 102	PC 105 (Cristal Global)	20nm, anatase

<sup>2</sup> Business and Industry Advisory Committee to the OECD: Representatives of industry within the OECD

<sup>3</sup> In the assessment of chemicals, an endpoint is the key parameter to be determined in a study, such as acute dermal toxicity or degradability in the environment.

<sup>4</sup> Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme [ENV/JM/MONO(2009)20/REV]: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2009\)20/rev&docLanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)20/rev&docLanguage=en)

<sup>5</sup> Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials [ENV/JM/MONO(2012)40]: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2012\)40&docLanguage=En](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2012)40&docLanguage=En)

TiO <sub>2</sub> NM acronym	Trade name (manufacturer)	properties (primary particle size in nanometres [nm], crystal structure, where applicable surface modification)
NM 103	UV Titan M262 (Sachtleben)	20nm, rutile, surface treatment: Al <sub>2</sub> O <sub>3</sub> and dimethicone, hydrophobic
NM 104	UV Titan M212 (Sachtleben)	20nm, rutile, surface treatment: Al <sub>2</sub> O <sub>3</sub> and glycerine, hydrophilic
NM 100 <sup>*</sup>	Tiona AT-1 (Cristal Global)	220nm, anatase

\* NM 105 is the “principal material”, i.e. the primary material for investigation in the context of the Sponsorship Programme on nTiO<sub>2</sub>.

<sup>\*</sup>NM 100, as a non-nanoscale TiO<sub>2</sub>, was chosen for comparison purposes.

The intention was to verify as many endpoints as possible for these materials, whereby a complete dataset for the above-mentioned points was if possible to be generated for NM 105 as the “principal material”. The data collation was to draw on scientific publications and involve the execution of experimental studies.

The experimental work on the OECD Sponsorship Programme was launched in 2009 and completed at the end of 2013. In the summer of 2014 the OECD decided to publish the WPMN results in IUCLID<sup>6</sup> dossiers. These dossiers contain both the collated data and information on the methods and protocols used in their collation. The dossiers are merely a compilation of the data and do not seek to evaluate them in terms of their relevance and quality for usability for regulation purposes. An assessment of the health or environmental risks of the investigated nanomaterials did not feature as an objective of the programme and was therefore not carried out. The published dossiers can be found on the Internet site of the OECD on the safety of nanomaterials (<http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm>).

## 2 Scientific results of the OECD Sponsorship Programme on nanoscale titanium dioxide with regard to environmental behaviour and ecotoxicology

Germany was responsible for the collation of data on ecotoxicology and environmental behaviour in the context of the testing programme for nTiO<sub>2</sub>. The majority of the data collated in Germany comes from projects funded by the environmental research plan (“Umweltforschungsplan”) of the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB) and supervised by the German Environment Agency. The next task in the OECD WPMN will then be to validate the data in terms of their quality and reliability for an assessment of the risks and the need for adjustment of the methods evaluated. In the following text, therefore, the German Environment Agency, acting by order of the BMUB, presents only a summary of the key scientific findings of the Sponsorship Programme in respect of the ecotoxicology and environmental behaviour of nTiO<sub>2</sub>. In order not to prejudice the results of the OECD, an assessment of the data does not take at this point.

The majority of the data collated on nTiO<sub>2</sub> in the OECD Sponsorship Programme refers to NM 105 as the “principal material”. Only partial data are available for the other variants.

<sup>6</sup> IUCLID = International Uniform Chemical Information Database; for more detailed information, please refer to the OECD site at: <http://www.oecd.org/chemicalsafety/risk-assessment/electronictoolsfordatasubmissionevaluationandexchange-intheoecdcooperativechemicalsassessmentprogramme.htm>

## 2.1 Scientific findings on the environmental behaviour of nTiO<sub>2</sub> nanomaterials

In the Sponsorship Programme, information was collated on the mobility and distribution behaviour of nTiO<sub>2</sub> in the environment. Since metal oxides, which also include nTiO<sub>2</sub>, are not subject to any biological or photo-catalytic degradation, these were not investigated. Aspects such as agglomeration, sedimentation and transformation, which are assumed to have effects on the further behaviour and bioavailability of nanomaterials in the environment, were however taken into account.

The nTiO<sub>2</sub> investigated in the test programme tend to agglomerate and settle in aqueous solution. This behaviour depends on the one hand on the properties of the nTiO<sub>2</sub> in question (e.g. size and surface properties) and, on the other hand, on the properties of the surrounding aqueous medium (salinity, pH, dissolved organic constituents).

Studies that simulate the retention of nTiO<sub>2</sub> (NM 105, NM 101) in sewage treatment plants have shown that a large proportion (>90%) of nTiO<sub>2</sub> is retained in sewage sludge and only a small proportion thereof finds its way into the treatment plant effluent.

A literature study on the mobility of nTiO<sub>2</sub> in water described that the transport of the nTiO<sub>2</sub> variants NM 101 and NM 105 in water is affected by constituents present in the water (such as films consisting of micro-organisms) which can bind to nanomaterials.

The low degree of mobility of the nTiO<sub>2</sub> under investigation (NM 105, NM 102 and NM 103) in soil was noted in column experiments with different soil types. A majority of the nTiO<sub>2</sub> introduced was found in the top soil layers, and only isolated agglomerations were shown to be present in the deeper soil segments.

No information on accumulation in organisms could be collated in the framework of the Sponsorship Programme. There are indications in the literature that nTiO<sub>2</sub> (NM105) can be transmitted down the food chain from one organism to another. There are also descriptions to the effect that nTiO<sub>2</sub> can affect the accumulation of other pollutants.

## 2.2 Scientific findings on the ecotoxicity of nTiO<sub>2</sub> nanomaterials

With regard to its toxic and ecotoxic effect, nTiO<sub>2</sub> is one of the most widely studied nanomaterials. The last 10 years have seen the publication of numerous scientific papers which offer an impression of the ecotoxic potential of this nanomaterial. These studies have however primarily concentrated on its acute effects on aquatic organisms. However, the number of studies on the ecotoxic effects on soil organisms and the long-term effects is slowly increasing. The Sponsorship Programme for nTiO<sub>2</sub> of the OECD WPMN has contributed to this trend.

### Ecotoxic effects on aquatic organisms

For fish, the majority of the available studies deal with short-term exposure. No mortality in adult fish could be determined in the present studies (tested were high concentrations of > 10 mg/l and > 100 mg/l). However, changes in tissues and organs and damage to gills have been described even at lower concentrations. Short-term exposure of fish embryos and larvae to nTiO<sub>2</sub> (NM 101, NM 102, NM 103, NM 104, NM 105) and to the non-nanoscale variant (NM 100) did not result in mortality at the tested concentrations. Studies looking at combined exposure to nTiO<sub>2</sub> and additional UV radiation simulating natural sunlight showed a significant increase in the mortality rate (investigated for 105 NM). No data collated according to standard test procedures on the long-term exposure of fish were made available in the context of the Sponsorship Programme for nTiO<sub>2</sub>.

To determine its ecotoxic effects on aquatic invertebrates, tests were carried out involving the short- and long-term exposure of small freshwater crustaceans to various nTiO<sub>2</sub> variants. Most of the studies

provided no evidence of mortality in the case of short-term exposure at the highest tested concentrations. Some studies did however reveal toxic effects at higher concentrations (>50mg/l). A significant increase in mortality was detected with an increase in exposure time and under the influence of the simulation of natural sunlight (NM 105, NM 101, NM 102, NM 100). The results of chronic exposure to nTiO<sub>2</sub> are contradictory: Some of the studies describe a lack of impact on reproduction and offspring. Others, however, describe an effect of NM 105 and NM 101. Furthermore, a study that considered exposure to nTiO<sub>2</sub> over several generations describes how NM 105 led to the reduction of reproductive success of daphnids and ultimately to the collapse of the community.

Studies on the exposure of fresh water algae to NM 105 or NM 101 show inhibition of growth. In addition, it has been found that nTiO<sub>2</sub> (105 NM) can accumulate in the algae. It was also revealed that the agglomeration of nTiO<sub>2</sub> can be associated with sedimentation of the algae.

A literature study describes how both NM 101 and NM 105 can cause damage to the cell membranes of aquatic microbial communities. These adverse effects were greater for unbound microorganisms than those living within the community of what is known as a biofilm.

The influence of nTiO<sub>2</sub> on the bacterial conversion of carbon compounds and ammonia that is exploited in wastewater treatment plants was the subject of a comparative study for NM 100, NM 101 and NM 102. No effect on microorganisms in the sludge was detected for any of the three TiO<sub>2</sub> types.

### **Ecotoxic effects on terrestrial and sediment-dwelling organisms**

In the Sponsorship Programme, data were collated on the effect of nTiO<sub>2</sub> on various plants, invertebrates and microorganisms living in soil or sediment.

Whereas NM105 demonstrated no harmful effect on carbon-converting micro-organisms, it did however have a slightly stimulating effect on those microorganisms which convert nitrogen. A literature study described the long-term effect of NM 105 on bacterial communities. It found that a high concentration of NM 105 (> 500 mg/kg soil) over 60 days led to a reduction in the respiration rate of the community occurring in natural soils.

To test the toxicity of nTiO<sub>2</sub> on the germination and the first growth of higher plants, the effects of exposure to NM 105 in various plants, including the oat, mustard, and mung bean, were tested over 14 days. No harmful changes were observed up to high test concentrations (100 or 1000 mg/kg).

Studies of the short- and long-term exposure of the earthworm to nTiO<sub>2</sub> revealed no harmful effect on survival and reproduction in the case of the various nTiO<sub>2</sub> variants under investigation (NM 105, NM 101, NM 102) or the non-nanoscale variant (NM 100). These findings are contradicted by a literature study which, in the case of the earthworm, describes a decline in cocoon production and the number of young animals after exposure to a similarly high concentration (1000 mg/kg soil) of NM 105.

The survival and reproduction of the predatory mite were not impaired by exposure to NM 105.

The potential ecotoxic effect of nTiO<sub>2</sub> was also examined in nematodes. After longer-term exposure to NM 105, effects on reproduction and the growth of the offspring (from concentrations of 10 mg/l) were detected in the test organism. No such harmful effects were detected in the case of the non-nanoscale reference, NM 100. Additional UV radiation simulating natural sunlight doubled the toxic effect of NM 105 on reproduction and growth. Absorption and accumulation in the intestinal tract were also investigated. The findings were that both NM 105 and the non-nanoscale reference NM 100 are absorbed by the intestinal tract, and that they form larger agglomerates in the intestine which prevent the further intake of food.

One ecotoxicological test organism of relevance to sediment is the blackworm. A study of the chronic effect of NM 105 after a period of exposure of 28 days detected no effects on the reproduction or biomass of the worms. Nor was any accumulation demonstrated in the organism.

## 2.3 Summary and Outlook

The data of the test programme for the various nTiO<sub>2</sub> variants show that properties of nanomaterials such as particle size, crystalline structure, surface properties and photo-activity influence their behaviour and ecotoxic effect in very complex ways. It therefore follows that a clear description of the nanomaterials on the basis of their physicochemical properties is important for a reliable interpretation of the data on their environmental behaviour and effect. The behaviour and the ecotoxic effect also depend on the surrounding environmental conditions and the sensitivity of the respective test organism. Ecotoxic effects of the nTiO<sub>2</sub> studied in the context of the Sponsorship Programme were detected in various tests with established test organisms, albeit mostly only at concentrations that are not currently expected in the environment.<sup>7</sup> UV radiation in the wavelength range of natural sunlight significantly increased the harmful effect of photo-active nTiO<sub>2</sub> and is relevant in particular for the investigation of harmful effects on aquatic organisms. Although large amounts of data were collated in the context of the Sponsorship Programme, gaps in the knowledge base remain. These include information on the long-term impact of nTiO<sub>2</sub> on fish and data on the accumulation in environmental organisms.

The next step is to assess the data collated in the OECD Sponsorship Programme in terms of their quality and conclusiveness for risk assessment and to identify the need for adjustment of the methods used. At the same time, it will also be necessary to look at possible differences in the environmental behaviour and effects of the different nTiO<sub>2</sub> variants. This will be part of the upcoming task of the OECD WPMN.

## 3 Need for adjustment of the methods used to record ecotoxicology and environmental behaviour in the OECD Sponsorship Programme for nanoscale titanium dioxide: Perspective of the German Environment Agency

Based on the preliminary findings of the OECD Sponsorship Programme the WPMN initiated a number of horizontal workshops which spanned various aspects (hence “horizontal”) of the safety analysis of nanomaterials. It was in this framework that well-known experts from more than 20 countries and organisations gathered in Berlin in early 2013 to discuss the applicability of selected existing OECD test methods for the identification of environmental behaviour and ecotoxicology to nanomaterials. They came to the conclusion that most of these test guidelines are in principle also applicable to nanomaterials. In many cases, however, adjustments in the form of guidance documents aimed specifically at nanomaterials are necessary. In addition, new test guidelines need to be developed to describe particular nano-specific aspects<sup>8</sup>.

---

<sup>7</sup> The choice of the concentration series used in the studies described here is based mostly on recommendations in the test guidelines which also cover the high concentration ranges in order to test for toxic effects. According to the current, even though provisional, assessment of nanomaterial concentrations in the environment, the concentrations in the environment fall well short of the concentrations currently tested.

<sup>8</sup> Expert Meeting Report: Ecotoxicology and Environmental fate of Manufactured Nanomaterials [ENV/JM/MONO(2014)1]: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2014\)1&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2014)1&doclanguage=en)



The general conclusion is that an adequate characterisation of nanomaterial in the respective test systems and a comprehensive analysis before, during and after the tests are essential for the reliable interpretation of the results. It is for this reason that specific provisions must be laid down in the context of the risk assessment for nanomaterials to determine what information is to be collated for analytical purposes in the framework of a test.

There is a specific need for adjustment in the form of a specific guidance document for all test guidelines dealing with the identification of ecotoxicological effects on aquatic organisms. These have certain freedoms in the test procedure that, while definitely justified for conventional chemicals, have the consequence when applied to nanomaterials that the results are comparable only with difficulty and are therefore insufficiently reliable. A working group formed in the aftermath of the workshop is currently elaborating a corresponding proposal. The guidance document to be developed will contain specifications on aspects such as a restrictive choice of test media, protocols for producing test suspensions and validation of the stability of the nanomaterials to be examined in the relevant test media for the duration of the test. Particularly when it comes to investigating the ecotoxicological effects of nTiO<sub>2</sub>, one has to take into consideration that, depending on their surface coating and crystal structure, their photocatalytic activity may differ. For this reason, it is essential that ecotoxicological tests carried out with aquatic organisms involve the use of simulated sunlight in order to reveal any increase in toxicity that may arise with light.

Knowledge of the agglomeration and dissolution behaviour of nanomaterials in the environment is an essential prerequisite for the further implementation of specific studies on the environmental behaviour and fate of nanomaterials in the environment, as well as for the interpretation of the results of these investigations. By means of both endpoints it is possible, for instance, to describe the forms in which nanomaterials are available in the various environmental compartments and what an appropriate testing strategy would look like. The agglomeration and dissolution behaviour of nanomaterials is subject to the surrounding environmental parameters, and both aspects in turn alter the behaviour and bioavailability of the nanomaterials. Two working groups are currently drafting two new test guidelines for the nano-specific collation of information on agglomeration and dissolution behaviour. In addition, a guidance document is being developed to describe how these two test guidelines can be jointly applied, with the aim of providing a decision-making aid for the appropriate implementation of further tests relating to environmental behaviour. This guidance document should also help with the interpretation of results from tests on environmental behaviour or ecotoxicology.

The existing methods for assessing the bioaccumulation of chemicals in aquatic organisms via the water phase, as used above all with fish<sup>9</sup>, cannot meaningfully be applied to the study of nanomaterials. This is primarily due to the fact that the conditions for the calculation of increasing concentrations in organisms (bioconcentration factor) are not fulfilled when applied to nanomaterials. Processes such as passive diffusion from the aqueous phase into the organism and equilibrium between water phase and organism are valid for soluble chemicals but not for particulate materials. It is for this reason that a working group is currently engaged in drafting a guidance document to describe which nano-specific aspects are to be taken into account when investigating the bioaccumulation in fish via intake through food. Moreover, the above-mentioned expert meeting found that the investigation of bioaccumulation and toxic effects on filtering organisms, especially sediment

---

<sup>9</sup> OECD 2012: OECD Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure. OECD Guidelines for the testing of chemicals, section 3. <http://www.oecd-ilibrary.org/docserver/download/9712191e.pdf?expires=1448378106&id=id&accname=guest&checksum=24606CA0A256005C2D99E6440BC3A516>. OECD Publishing, Paris.

inhabitants, is of great importance due to the particular structure of nanomaterials and their tendency to agglomeration and sedimentation.

The existing test guidelines for the determination of absorption and desorption processes for chemicals in soil are not applicable to nanomaterials<sup>10</sup>. It is not possible with this method to make a distinction between agglomerated nanomaterials and those adsorbed in soil. Here a new test method for nanomaterials is required. For a better understanding about the behaviour of nanomaterials in soil a nano-specific guidance document is currently being drafted for the test guidelines on leaching in soil columns<sup>11</sup>.

Nanomaterials are subject to physicochemical changes in the course of time and in dependence on the characteristics of the surrounding environment. This “ageing” process has a key role to play in the proper assessment of the environmental behaviour of nanomaterials. A standardised protocol which describes the investigation of chemical changes in nanomaterials in the environment, taking into account the essential processes, should therefore be developed and included in the assessment of environmental behaviour and effects. Aspects that should feature in this protocol include the loss of surface coatings in addition to chemical changes brought about by oxidation or reduction.

Table 2: Overview of the need for revision and supplementation of the OECD test guidelines used in the Sponsorship Programme for the areas of environmental behaviour and ecotoxicology

	Need for adjustments to determine environmental behaviour	Need for adjustments to determine ecotoxicology	Superordinate need for adjustments
<b>Test guidelines and guidance documents for the aquatic environment</b>	Test guidelines and guidance documents for the description of the agglomeration behaviour and dissolution rate of NM* in different aquatic media  Nano-specific guidance document on bioaccumulation in fish after intake via food	Guidance documents for dealing with NM in the existing guidelines for the detection of their effects in aquatic organisms	Instructions on the physicochemical characterisation of NM  Instructions on the investigation of chemical changes in NM in the environment over time
<b>Test guidelines and guidance documents for</b>	New test guidelines to describe the desorption and adsorption of NM in soil	Instructions on the application of NM into the test soil/sediment	

<sup>10</sup> OECD 2000: OECD Test No. 106: Adsorption – Desorption Using a Batch Equilibrium Method. OECD Guidelines for the testing of chemicals, section 1. <http://www.oecd-ilibrary.org/docserver/download/9710601e.pdf?expires=1448378244&id=id&accname=guest&checksum=B0CB0F3F208F980CEBEE90B885A67FF2>. OECD Publishing, Paris.

<sup>11</sup> OECD 2004: OECD Test No. 312: Leaching in Soil Columns. OECD Guidelines for the testing of chemicals, section 3. <http://www.oecd-ilibrary.org/docserver/download/9731201e.pdf?expires=1448378491&id=id&accname=guest&checksum=940EAFE6658403ECE5D38267ADE45230>. OECD Publishing, Paris.

	Need for adjustments to determine environmental behaviour	Need for adjustments to determine ecotoxicology	Superordinate need for adjustments
<b>soil and sediment</b>	Nano-specific guidance document on leaching in soil	Instructions on the analysis of NM in soil	

\* NM = nanomaterial

## 4 Research needs in the field of ecotoxicology and environmental behaviour identified on the basis of the results of the OECD Sponsorship Programme

It is the belief of the German Environment Agency that the following further research themes should be pursued on the basis of the findings of the OECD Sponsorship Programme:

- Studies on the ageing of nanomaterials under varying environmental conditions in the various environmental compartments (water, soil, air, sediment, but also more specific compartments such as sewage sludge) over time and their influence on the acute and long-term effects on environmental organisms
- Studies on the accumulation of nanomaterials in environmental organisms
- Studies on the long-term effects of nanomaterials in fish
- Development of criteria for the grouping of nanomaterials for a substantiated transfer of data on environmental behaviour and ecotoxicology between forms of nanomaterials or between nanomaterials and the macroscopic form

A significant research need above and beyond the actual objectives of the OECD Sponsorship Programme is the development of valid models to assess the environmental exposure of nanomaterials. To this end it will be necessary to establish relations between the knowledge of production quantities, areas of use, possible means of release and quantities of materials released, and the specific behaviour of nanomaterials in the environmental compartments. A reliable estimation of the environmental concentrations of nanomaterials is essential in order to assess the relevance of the effect concentrations found.

### Acknowledgements

The German delegation to the OECD WPMN would like to express its thanks to all the participating national and international research institutions which have supported and provided data for the nTiO<sub>2</sub> Sponsorship Programme:

Austria:

- University of Vienna, Department of Environmental Geosciences
- University of Graz, Institute of Pharmaceutical Sciences

Canada:

- McGill University, Department of Chemical Engineering,
- National Research Council, Biotechnology Research Institute, Applied Ecotoxicology Group
- Trent University, Department of Environmental & Resource Studies
- Environment Canada, Environmental Health Science and Research Bureau
- Health Canada, Healthy Environments & Consumer Safety Branch



- Wilfrid Laurier University, Institute for Water Science
- University of Victoria, Department of Biochemistry and Microbiology
- HydroQual Laboratories
- University of Alberta, Biological Sciences

Denmark:

- Technical University of Denmark (DTU), Department of Environmental Engineering

France (EU FP7 research project "NanoGenotox"):

- French Agency for Food, Environmental and Occupational Health & Safety, Unit of Toxicology of Contaminants, Department of Information, Communication and Dialogue with Society, Unit of European and International Affairs (ANSES)
- Federal Institute for Risk Assessment, (BfR, Germany)
- French Atomic Energy Commission, Materials Sciences Division, Life Sciences Division (CEA)
- Veterinary and Agrochemical Research Centre, Unit of Electron Microscopy (CODA-CERVA, Belgium)
- Joint Research Centre, Institute for Health and Consumer Protection (IHCP), Nanotechnology (EC/JRC-IHCP)
- Finnish Institute of Occupational Health, New Technologies and Risks laboratory (FIOH)
- Roumen Tsanev Institute of Molecular Biology Bulgarian Academy of Sciences, Medical and Biological Research Laboratory, Institute of Mineralogy and Crystallography Bulgarian Academy of Sciences, Central Laboratory of Mineralogy (Bulgaria)
- Medical and Biological Research Laboratory, Aerosol Metrology Laboratory and the Inorganic Analysis and Aerosol Characterization Laboratory, Carcinogenesis and Developmental Toxicology Laboratory, Pollutants and Health Department (INRS)
- National Health Institute Doutor Ricardo Jorge, Genetic Toxicology R&D Unit (INSA, Portugal)
- Scientific Institute of Public Health, Laboratory of toxicology (IPH, Belgium)
- Institut Pasteur of Lille, Genetic Toxicology Laboratory (IPL, France)
- Istituto Superior di Sanita, Food and Veterinary Toxicology Unit (ISS, Italy)
- Laboratoire national de métrologie et d'essais (LNE, France)
- The Nofer Institute of Occupational Medicine (Poland), Laboratory of Molecular Toxicology (NIOM, Poland)
- National Research Centre for the Working Environment, Nanotoxicology and Occupational Hygiene Group (NRCWE, Denmark)
- National Institute for Public Health and the Environment, The Laboratory for Health Protection Research (RIVM, the Netherlands)
- Universitat Autònoma de Barcelona, Group of Mutagenesis, (UAB, Spain)

Germany:

- Institute of Energy and Environmental Technology e.V. (IUTA), Air quality control and Nanotechnology
- Fraunhofer Institute for Toxicology & Experimental Medicine (ITEM)
- Fraunhofer Institute for Molecular Biology and Applied Ecology (IME)
- RWTH Aachen, Institute for Environmental Research
- Goethe University Frankfurt (Main), Institute for Ecology, Evolution and Diversity
- Technische Universität Dresden, Institute of Process Engineering and Environmental Technology
- Hamburg University of Applied Sciences, Hazardous Materials and Environmental Toxicology
- BAM, Federal Institute for Materials Research and Testing

Japan:

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

Korea:

- Dongduk Women's University, College of Pharmacy
- Hanyang University, Laboratory of Nanoscale Characterisation & Environmental Chemistry
- Korea Research Institute of Standards and Science, Korea Research Institute of Standards and Science Division of Industrial Metrology
- Seoul National University, School of Chemical & Biological Engineering
- Kyung Hee University, Department of Applied Chemistry
- Korea University, School of Life Science & Biotechnology

Spain:

- INIA, Departamento de Medio Ambiente

USA:

- National Institute for Standards and Technology (NIST), Nanoparticle Measurements & Standards
- Environmental Protection Agency (EPA), National Health and Environmental Effects Research
- Environmental Protection Agency (EPA), Ecology Division
- Food and Drug Administration (FDA), National Center for Toxicological Research

The German delegation to the OECD WPMN is also grateful to the French partner ANSES, to the Titanium Dioxide Manufacturers Association (TDMA) for providing nTiO<sub>2</sub> samples, and to the Joint Research Centre (JRC) of the EU for preparation and distribution of the samples.