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PRELIMINARY GUIDANCE NOTES ON NANOMATERIALS: INTERSPECIES VARIABILITY FACTORS IN HUMAN HEALTH RISK ASSESSMENT

Series on the Safety of Manufactured Nanomaterials
No. 58

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PRELIMINARY GUIDANCE NOTES ON NANOMATERIALS: INTERSPECIES VARIABILITY FACTORS IN HUMAN HEALTH RISK ASSESSMENT

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
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FOREWORD

The OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) held a Special Session on the Potential Implications of Manufactured Nanomaterials for Human Health and Environmental Safety (June 2005). This was the first opportunity for OECD member countries, together with observers and invited experts, to begin to identify human health and environmental safety related aspects of manufactured nanomaterials. The scope of this session was intended to address the chemicals sector.

As a follow-up, the Joint Meeting decided to hold a Workshop on the Safety of Manufactured Nanomaterials in December 2005, in Washington, D.C. The main objective was to determine the "state of the art" for the safety assessment of manufactured nanomaterials with a particular focus on identifying future needs for risk assessment within a regulatory context.

Based on the conclusions and recommendations of the Workshop [ENV/JM/MONO(2006)19] it was recognised as essential to ensure the efficient assessment of manufactured nanomaterials so as to avoid adverse effects from the use of these materials in the short, medium and longer term. With this in mind, the OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee in September 2006. This programme concentrates on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. This programme promotes international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials.

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

PRELIMINARY GUIDANCE NOTES ON NANOMATERIALS: INTERSPECIES VARIABILITY FACTORS IN HUMAN HEALTH RISK ASSESSMENT

BACKGROUND

1. The document Report on Risk Assessment of Manufactured Nanomaterials – Important Issues was endorsed at the 9th meeting of the Working Party on Manufactured Nanomaterials and has been published in March 2012 (ENV/JM/MONO(2012)8). Based on this report and according to the conclusions (endorsed by the Chemicals Committee at its 48th meeting in February 2012) of the Mid-term review of the WPMN programme, Steering Group 6 (SG6, Co-operation on Risk Assessment) initiated a survey to identify high priority issues in risk assessment of nanomaterials for which SG6 shall aim to produce practical guidance (ENV/JM/MONO(2013)18). It was agreed that SG6 Projects should be initiated in order to address priority issues once the survey analysis is finalised. Furthermore, it was agreed that two Pilot Projects should be initiated with the aim to collect relevant expertise in the particular environment of the WPMN. Pilot Project 1 was initiated in August 2012 with the objective to review the established default uncertainty / safety / assessment factors for chemicals currently used for human health risk characterisation in light of the data gathered in the OECD WPMN Sponsorship Programme. The objectives of the Pilot Project also included a gap analysis of data required to produce the specific guidance, providing support to WPMN in planning of a potential second Testing Programme, as well as collecting experience with the use of the reporting format (the JRC NANOhub) used in the Sponsorship Programme. Interim reports were presented for discussion in SG6 which was merged becoming SGAP (Risk Assessment and Regulatory Programmes) and WPMN at their meetings in June 2013 (SG6) and December 2013 (SG-AP). Part of the project was presented at the OECD WPMN Expert Meeting on Toxicokinetics (26-28th February, 2014) in Seoul, South Korea.

INTRODUCTION

2. In order to derive health based Reference Values from the results of toxicological studies, assessment factors - also termed safety or uncertainty factors - are applied to a relevant starting point (or point of departure) which can be a NOAEL[1] or a BMDL5[2] observed in the particular study. At the Workshop on Risk Assessment of Manufactured Nanomaterials in a Regulatory Context (16-18th September, 2009) in Washington D.C., United States of America, it was concluded, following discussion, that the application of standard uncertainty factors applied in the assessment of chemicals to the risk assessment of nanomaterials should undergo validation and that a justification should be provided when using uncertainty factors currently not validated for nanomaterials (ENV/JM/MONO(2010)10).

3. Follow-up discussion documented in the OECD WPMN Report Important Issues on Risk Assessment of Manufactured Nanomaterials confirmed that "*the derivation of a RfD or any other exposure limit from effect levels like NOAELs requires knowledge of the appropriate Assessment Factors (AFs) to account for variability and uncertainty in the risk estimates*". With regard to extrapolation of toxicity data between species, it was stated that the existing standard (default) assessment factors were established based on extensive historical knowledge, however, their appropriateness for the derivation of exposure limits for nanomaterials requires further research considering potential differences in extent and pattern of

[1] No Observed Adverse Effect Level, the highest tested dose at which no adverse effect was observed.

[2] 5% Benchmark Dose Lower Confidence Limit, the lower limit of a one-sided 95% confidence interval on the 5 % benchmark dose (BMD) - the computed exposure associated with a 5% incidence of the adverse effect.

deposition, clearance mechanisms and capacity, or sensitivity. Concerning uncertainty in and variability of toxicological response within species, the report noted that this would currently not be sufficiently documented for nanomaterials. In particular, it was reasoned that vertebrate toxicity tests as used in human health hazard assessment mostly employ minimum numbers of animals with low genetic variability, which further hampers gaining insight in intraspecies variability (ENV/JM/MONO(2012)8).

4. Finally, in a prioritisation exercise, the issues of intra- and interspecies extrapolation in human health risk assessment covering the discussion described above, received medium-level scores for likely data availability and for importance to regulatory risk assessment (ENV/JM/MONO(2013)18).

5. Therefore, the subject was selected for a first Pilot Project to support future development of Guidance Notes on Risk Assessment of Nanomaterials by the Steering Group Risk Assessment and Regulatory Programmes (SG-AP) of OECD WPMN. The detailed objective included to:

- identify and review studies from the Sponsorship Programme and the published literature addressing the toxicity of nanomaterials included in the WPMN Testing Programme in different mammalian species,
- identify and review studies addressing the toxicity of similar nanomaterials in different mammalian species,
- identify and review suitable historic (human) data,
- identify suitable toxicokinetic data for nanomaterials in different mammalian species,
- perform predictions of toxicokinetic behaviour of hypothetic nanomaterials in rat and humans (limited to inhalation exposures, using the Multiple Path Particle Dosimetry (*MPPD*) *model*[3],
- compare and discuss observed differences in susceptibility / kinetics between species to established default variability factors,
- make recommendations on how to improve the database, if required, for such comparative analysis.

The expected outcome included:

- a preliminary analysis of the applicability of established default variability factors (also uncertainty, safety, or assessment factors) for characterisation of risks to human health from nanomaterials, supporting the development of further guidance (report),
- an enhanced interaction with the WPMN Sponsorship Programme and the development of modes of access to data from the Programme,
- a data gap analysis as a basis for recommendations of SG-AP to WPMN indicating needs for further experimental work as applicable.

A Master´s thesis and an Expert Opinion were initiated to meet the objectives outlined above.

[3] The Multiple-Path Particle Dosimetry (MPPD) model is a computational model that can be used for estimating human and rat airway particle dosimetry.

METHODOLOGY

6. Manufactured nanomaterials included in the scope were zinc oxide (ZnO), cerium (IV) oxide (CeO_2), silicon dioxide (SiO_2), silver (Ag), gold (Au), single-walled carbon nanotubes (SWCNT), multi-walled carbon nanotubes (MWCNT), titanium dioxide (TiO_2), iron (III) oxide (Fe_2O_3) and nanoclay. Relevant information on toxicity following repeated dosing was obtained from the (draft) dossiers of the OECD WPMN Sponsorship Programme and the published literature as available until August 2013. The PubMed database was used for literature searches and relevant publications were selected in three steps taking into account test species, test material and exposure scheme.

7. A total of 112 relevant repeated-dose toxicity studies were identified. The majority of these studies were conducted using rats (60 studies) and mice (44 studies) with exposure by inhalation or through the oral route. There were few studies with exposure by dermal application. Eight studies on species other than rat and mouse were available (rabbits: 1, hamsters: 2, guinea pigs: 1, pigs: 4). A limited number of studies involved subchronic or chronic exposure (34 subchronic, 4 chronic).

8. The database of comparable repeated-dose toxicity studies on all analysed nanomaterials in different species was insufficient to draw final conclusions about interspecies variability in sensitivity towards nanomaterial toxicity between laboratory animals. In particular, there was a lack of studies with identical nanomaterial across different species. Therefore, it was difficult to discriminate between possible interspecies variability and variations due to differences in material properties, impurities, or vehicle effects. However, there were indications for variability in studies with different species exceeding a factor of ten for TiO_2 nanomaterials as well as for other nanomaterials, namely SiO_2 and silver. As the origin of these larger differences may be attributed to a range of causes, these results should be considered as indicative and would need to be followed-up by repeated-dose toxicity studies performed under comparable conditions and with identical material in different species.

9. In addition, it was attempted to elaborate potential species differences between rat and humans with regard to deposition in and clearance from the respiratory tract following inhalation of nanomaterials using the modelling software MPPD. In a first step, the predictivity of the software was assessed by comparing experimental data identified from the Sponsorship Programme and the published literature as described above. Based on the results of the analysis, this approach was discontinued.

RESULTS & DISCUSSION

1. Intraspecies Variability in Repeated-Dose Toxicity Studies

10. Individual animal data was not accessible. In addition, studies that were performed in different strains of one species with identical material and comparable exposure conditions could not be identified. Therefore, an analysis for intraspecies variability was not performed.

2. Interspecies Variability in Repeated-Dose Toxicity Studies

11. Information from repeated-dose toxicity studies in different species obtained from WPMN draft dossiers and published literature was analysed separately for each material class:

C60 fullerene, Single-walled carbon nanotubes, Zinc oxide, Gold, Iron (III) oxide, Nanoclay:

12. Due to a general lack of studies performed under comparable exposure conditions but in different species, the extent of interspecies variation was not analysed for these nanomaterials.

Cerium(IV) oxide:

13. Three relevant studies in rats and mice, respectively, were identified.

Study No.	Material, Description	Species, Strain, Sex and No. per dose	Exposure route, Duration, (Postexposure)	Doses	NOAEL / LOAEL
8	CeO_2 NM-213	Rat Wistar 5m + 5f	Inhalation (nose-only) 32 to 33 days (+28 days)	Equiv. to 0 / 5.9 / 18.7 / 55 mg/m³ x 6 h	NOAEL: not determined LOAEL: 5.9 mg/m³
9	CeO_2 NM-211	Rat Wistar 5m + 5f	Inhalation (nose-only) 28 days (+28 days)	Equiv. to 0 / 1.2 / 3.5 / 10.8 mg/m³ x 6 h	NOAEL: not determined LOAEL: 1.2 mg/m³
10	CeO_2 NM-212	Rat Wistar 5m + 5f	Inhalation (nose-only) 30 days (+ 28 days)	Equiv. to 0 / 2.5 / 6.7 / 19.9 mg/m³ x 6 h	NOAEL: not determined LOAEL: 2.5 mg/m³
11	CeO_2 Envirox	Mouse ApoE-/- 5m + 5f	Inhalation (nose-only) 28 days (+ 17 days)	Equiv. to 0 / 0.9 / 0.57 / 1.7 mg/m³ x 3 h	NOAEL: 1.7 mg/m³ x 3 h (eq. to 0.85 mg/m³ x 6 h) LOAEL: not determined
12	CeO_2 15-30 nm diameter N&M Inc.	Mouse CD-1 18 male	Inhalation (nose-only) 28 days (+ 14 and 28 days)	0 / 2 mg/m³ x 6 h	NOAEL: not determined LOAEL: 2 mg/m³
13	CeO_2 15-30 nm diameter N&M Inc.	Mouse CD-1 18 male	Inhalation (nose-only) 14 days (+ 14 days)	0 / 2 mg/m³ x 6 h	NOAEL: not determined LOAEL: 2 mg/m³

14. Interspecies variability was not analysed as studies were performed with different materials and description of the dose response relationship was incomplete (only LOAELs determined except for the ApoE-/- mouse model).

Silicon Dioxide:

15. Six relevant studies in rats, two in mice, one in rabbits and one in hamsters with exposure by oral administration were available for silicon dioxide nanomaterials. No relevant effects were observed in rats up to a dose of 10 % in feed or 1600 mg/kg bw/day per gavage in rabbits and hamsters. Studies were

conducted partly with the same nanomaterial (NM-204). One study summary indicated relevant liver toxicity by oral administration in mice but a full study report was not available for review. Furthermore, a different nanomaterial was used. Therefore, the suspected toxicity could have resulted from impurities or coating of the silica or differences between silicas. Confirmation should be sought. No statement can be given on interspecies variability of silicon dioxide nanomaterials toxicity based on the available data.

Silver:

16. For silver nanomaterials there were six and four repeated-dose toxicity studies available for rats and mice, respectively. In mice, LOAELs were reported at a dose of 1 mg/kg bw/day. One toxicity study determined a NOAEL of 0.5 mg/kg bw/day in mice. The lowest LOAEL in rats was 125 mg/kg bw/day (90-day exposure). Effects observed in rats were changes in haematology, clinical chemistry as well as histopathological changes in the liver (e.g. hyperplasia, necrosis). No changes were observed in kidney or other organs. In mice, effects were observed in clinical chemistry, cytokine expression, and changes in phenotypes of immune cells as well as cell infiltration in kidneys. Taking into account that the silver nanomaterials in the mice studies were modified with tetrahydrofurane[4] (THF), and target organs in rats and mice were dissimilar, no definitive statement can be given on interspecies variability of silver nanomaterials by oral administration.

17. Two studies were available with dermal exposure in guinea pigs and pigs. Nanomaterials used were dissimilar in these studies. Furthermore, no NOAEL was determined in either study. Therefore, no statement can be given on interspecies variability of silver nanomaterials by dermal application.

Study No.	**Material, Description**	**Species, Strain, Sex and No. per dose**	**Exposure route, Duration, (Postexposure)**	**Doses**	**NOAEL / LOAEL**
43	n-Ag 18 nm diameter (in-house synthesis)	Rat SD 10m + 10f	Inhalation (whole body) 90 days	0 / 49 / 133 / 515 µg/m³ x 6 h	NOAEL: 133 µg/m³ LOAEL: 515 µg/m³
44	n-Ag 12.6-15.4 nm diameter (in-house synthesis)	Rat SD 5m + 5f	Inhalation (whole body) 28 days	0 / 0.48 / 3.48 / 61.24 µg/m³ x 6 h	NOAEL: 3.48 µg/m³ LOAEL: 61.24 µg/m³
45	n-Ag 10 nm diameter (N&M Inc.)	Mouse C57BL/6 13 male	Inhalation (whole body) 12 days (+ 3 weeks)	0 / 3.3 µg/m³ x 4 h	NOEL: not determined LOEL: 3.3 µg/m³

[4] a substance classified for human health endpoints as eye irritant, Specific Target Organ Toxicity - Single Exposure (STOT-SE) 3, carcinogen class 2 according to the United Nations Globally Harmonised System for classification and labelling.

46	n-Ag 56 nm diameter (NAMATECH)	Rat Fisher 344 5m + 5f	Oral (gavage) 90 days	0 / 30 / 125 / 500 mg/kg bw/day	NOAEL: 30 mg/kg bw/day LOAEL: 125 mg/kg bw/day
49	n-Ag 14 nm diameter (in-house synthesis)	Rat Wistar Con. and HD: 6m+10f LD and MD: 8 female	Oral (gavage) 28 days	0 / 2.25 / 4.5 / 9.0 mg/kg bw/day	NOAEL: 9.0 mg/kg bw/day LOAEL: not determined
50	n-Ag 60 nm diameter (NAMATECH)	Rat SD 5m + 5f	Oral (gavage) 28 days	0 / 30 / 300 / 1000 mg/kg bw/day	NOAEL: 30 mg/kg bw/day LOAEL: 300 mg/kg bw/day
51	n-Ag <20 nm diameter citrate capped (ABC Nanotech)	Rat SD 50m + 50f	Oral (gavage) 42 to 52 days (+ 14 to 16 days) Reproduction / Developmental Toxicity Screening	0 / 62.5 / 125 / 250 mg/kg bw/day	NOAEL: ≥ 125 mg/kg bw/day LOAEL: ≥ 250 mg/kg bw/day
52	n-Ag 42 nm diameter (Sigma-Aldrich) Surface coated with THF	Mouse ICR 6m + 6f	Oral (gavage) 28 days	0 / 0.25 / 0.5 / 1 mg/kg bw/day	NOAEL: 0.5 mg/kg bw/day LOAEL: 1 mg/kg bw/day
53	n-Ag 22 nm diameter (Sigma-Aldrich) Surface coated with THF	Mouse ICR 5m + 5f	Oral (gavage) 14 days	0 / 1 mg/kg bw/day	NOEL: not determined LOEL: 1 mg/kg bw/day
54	n-Ag 42 nm diameter (Sigma-Aldrich) Surface coated with THF	Mouse ICR 5m + 5f	Oral (gavage) 14 days	0 / 1 mg/kg bw/day	NOEL: not determined LOEL: 1 mg/kg bw/day
55	n-Ag 71 nm diameter (Sigma-Aldrich) Surface coated with THF	Mouse ICR 5m + 5f	Oral (gavage) 14 days	0 / 1 mg/kg bw/day	NOEL: not determined LOEL: 1 mg/kg bw/day

Multi-walled carbon nanotubes:

18. No repeated-dose toxicity data was available for dermal application of MWCNT and only two studies in rats were available for repeated-dose toxicity following oral exposure. Therefore, no statement on interspecies variability was be given for these routes of exposure. For exposure by inhalation, seven relevant studies were available in rats and five in mice. No subchronic toxicity study was available for mice, no chronic toxicity study was available for either species and no toxicity studies were available with other species for exposure by inhalation. Variability in the toxicity of different MWCNT has been

observed in rats with exposure by inhalation. For example a LOAEL at a dose of 0.1 mg/m³ (NOAEL not identified) has been reported for Nanocyl-MWCNT, but a NOAEL at a dose of 0.1 mg/m³ with a LOAEL at a dose of 0.4 mg/m³ was observed for Baytubes-MWCNT after 90-days of exposure, respectively. Both studies were OECD guideline studies conducted under GLP criteria. In mice, only one study was able to determine a NOAEL at a dose of 0.3 mg/m^3 and a LOAEL at a dose of 1.0 mg/m³. However, this study exposed the test animals for 14 days only. No haematology or clinical chemistry was conducted in this study. Thresholds were based on changes in T-cell-dependent antigen response and cytokine excretion in spleen. Because of the short study duration, different endpoints analysed as well as different nanomaterials used, no definitive statement on interspecies variability of MWCNT with exposure by inhalation was made.

Study No.	**Material, Description**	**Species, Strain, Sex and No. per dose**	**Exposure route, Duration, (Postexposure)**	**Doses**	**NOAEL / LOAEL**
70	MWCNT 5-15 nm diameter (Nanocyl)	Rat Wistar 10m + 10f	Inhalation (nose-only) 90 days	0 / 0.1 / 0.5 / 2.5 mg/m³ x 6 h	NOAEL: not determined LOAEL: 0.1 mg/m³
71	MWCNT 10 nm diameter (Baytubes)	Rat Wistar 10m + 10f	Inhalation (nose-only) 90 days (+ 4, 13 and 26 weeks)	0 / 0.1 / 0.4 / 1.5 / 6.0 mg/m³ x 6 h	NOAEL: 0.1 mg/m³ LOAEL: 0.4 mg/m³
72	MWCNT (Hodogaya)	Rat Fischer 344 10m + 10f	Inhalation (whole body) 90 days	0 / 0.2 / 1.0 / 5.0 mg/m³ x 6 h	NOAEL: not determined LOAEL: 0.2 mg/m³
73	MWCNT 44 nm diameter (Nikkiso)	Rat Wistar 5 male	Inhalation (whole body) 28 days (+ 3 days, 1 and 3 month)	0 / 0.37 mg/m³ x 6 h	NOAEL: not determined LOAEL: 0.37 mg/m³
74	MWCNT 88 nm diameter (Hodogaya)	Rat Fischer 344 5m + 5f	Inhalation (whole body) 14 days (+ 4 weeks)	0 / 0.2 / 1.0 / 5.0 mg/m³ x 6 h	NOAEL: 0.2 mg/m³ LOAEL: 1.0 mg/m³
75	MWCNT (Arkema)	Rat Wistar 20m + 10f	Inhalation (nose-only) 5 days (+ 4 weeks)	0 / 0.066 / 0.26 / 1.3 mg/m³ x 6 h	NOAEL: 0.26 mg/m³ LOAEL: 1.3 mg/m³
76	MWCNT (Arkema)	Rat Wistar 11 male	Inhalation (nose-only) 5 days (+ 21 weeks)	0 / 0.15 / 0.57 / 2.86 mg/m³ x 6 h	NOAEL: 0.15 mg/m³ LOAEL: 0.57 mg/m³

Study No.	Material, Description	Species, Strain, Sex and No. per dose	Exposure route, Duration, (Postexposure)	Doses	NOAEL / LOAEL
77	MWCNT 50 nm diameter (Shenzhen)	Mouse Kunming 9 female	Inhalation (whole body) 60 days	Mean concentration: 0 and 32.6 mg/m³ x 6 h	NOAEL: not determined LOAEL: 32.6 m³
78	MWCNT 50 nm diameter (Shenzhen)	Mouse Kunming 9 female	Inhalation (whole body) 30 days	Mean concentration: 0 and 32.6 mg/m³ x 6 h	NOAEL: 32.6 mg/m³ LOAEL: not determined
79	MWCNT 10-20 nm diameter (Shenzhen)	Mouse C57BL/6 6 male	Inhalation (whole body) 14 days	0 / 0.3 / 1.0 / 5.0 mg/m³ x 6 h	NOAEL: 0.3 mg/m³ LOAEL: 1.0 mg/m³
80	MWCNT 10-20 nm diameter (Shenzhen)	Mouse C57BL/6 6 male	Inhalation (whole body) 7 days	0 / 0.3 / 1.0 / 5.0 mg/m³ x 6 h	NOAEL: 5.0 mg/m³ LOAEL: not determined
81	MWNCT 20-50 nm diameter (Sigma)	Mouse BALB/c 6 male	Inhalation (nose-only) 7 days	0 / 5 mg/kg bw/day x 20 min	NOAEL: not determined LOAEL: 5 mg/kg bw
82	MWCNT 30 nm diameter (Nikkiso)	Rat SD 6m + 6f	Oral (gavage) 28 days (+ 14 days)	0 / 0.5 / 5 / 50 mg/kg bw/day	NOAEL: 50 mg/kg bw/day LOAEL: not determined
83	MWCNT 10-15 nm diameter (Hanwha)	Rat SD 12 pregnant females	Oral (gavage) GD 6 to 19 (developmental toxicity)	0 / 40 / 200 / 1000 mg/kg bw/day	Dams and Developmental: NOAEL: 200 mg/kg bw/day LOAEL: 1000 mg/kg bw/day

Titanium Dioxide:

19. Repeated-dose toxicity studies with exposure to TiO_2 by inhalation indicated significant interspecies variability. Rats appeared to be the more vulnerable species compared to mice and hamsters. The interspecies variability may be described with a factor of four to five based on a 90-day whole body inhalation toxicity study conducted by Bermudez et al. with rats, mice and hamsters (Studies no. 85, 94 and 96, respectively).

Study No.	Material, Description	Species, Strain, Sex and No. per dose	Exposure route, Duration, (Postexposure)	Doses	NOAEL / LOAEL
84	TiO_2 P25 (Degussa)	Rat Wistar 288 female	Inhalation (whole body) 24 month	0 / 10 mg/m³ x 18 h	NOAEL: not determined LOAEL: 10 mg/m³
85	Uf-TiO_2 P25 (Degussa)	Rat Fischer 344 25 female	Inhalation (whole body) 90 days (+ 4, 13, 26 and 52 weeks)	0 / 0.52 / 2.1 / 10.5 mg/m³ x 6 h	NOAEL: 0.52 mg/m³ LOAEL: 2.1 mg/m³
86	TiO_2 P25 (Degussa)	Rat Fischer 344 64m (in total)	Inhalation (whole body) 12 weeks (up to 6 month)	0 / 23.5 mg/m³ x 6 h	NOAEL: not determined LOAEL: 23.5 mg/m³
87	TiO_2 NM-103	Rat Wistar 12 male	Inhalation (nose-only) 28 days (+ 3, 45 and 94 days)	0 / 3.1 / 12.1 / 49.7 mg/m³ x 6 h	NOAEL: 3.1 mg/m³ LOAEL: 12.1 mg/m³
88	TiO_2 NM-104	Rat Wistar 12 male	Inhalation (nose-only) 28 days (+ 3, 45 and 94 days)	0 / 3.2 / 12.2 / 47.7 mg/m³ x 6 h	NOAEL: 3.1 mg/m³ LOAEL: 12.1 mg/m³
89	TiO_2 NM-105	Rat Wistar 12 male	Inhalation (nose-only) 28 days (+ 3, 45 and 94 days)	0 / 3.2 / 12.3 / 47.7 mg/m³ x 6 h	NOAEL: 3.1 mg/m³ LOAEL: 12.1 mg/m³
90	TiO_2 P25 (Evonik)	Rat	Inhalation 21 days (+ 3, 28 and 90 days)	0 / 2 / 10 mg/m³ x 6 h	NOAEL: 2 mg/m³ LOAEL: 10 mg/m³
91	TiO_2 20-30 nm diameter	Rat Wistar 12 male	Inhalation (nose-only) 5 days (+ 3 and 14 days)	0 / 88 mg/m³ x 6 h	NOAEL: not determined LOAEL: 88 mg/m³
92	TiO_2 25.1 nm diameter (Baker)	Rat Wistar 14 male	Inhalation (nose-only) 5 days (+ 3 and 16 days)	0 / 2 / 10 / 50 mg/m³ x 6 h	NOAEL: not determined LOAEL: 2 mg/m

Study No.	Material, Description	Species, Strain, Sex and No. per dose	Exposure route, Duration, (Postexposure)	Doses	NOAEL / LOAEL
93	TiO_2 P25 (Degussa)	Mouse NMRI 160 female	Inhalation (whole body) 13.5 months	0 / 10 mg/m³ x 18 h	NOAEL: not determined LOAEL: 10 mg/m³
94	TiO_2 P25 (Degussa)	Mouse B6C3F1 25 female	Inhalation (whole body) 90 days (+ 4, 13,26 and 52 weeks)	0 / 0.54 / 2.2 / 10.8 mg/m³ x 6 h	NOAEL: 2.2 mg/m³ LOAEL: 10.8 mg/m³
95	TiO_2 5 nm diameter (N&M Inc.)	Mouse C57B1/6 6 male	Inhalation (whole body) 10 days (+ 1, 2 and 3 weeks)	0 / 8.88 mg/m³ x 4 h	NOAEL: 8.88 mg/m³ LOAEL: not determined
96	Uf-TiO_2 P25 (Degussa)	Hamster Syrian 25 female	Inhalation (whole body) 90 days (+ 4, 13,26 and 52 weeks)	0 / 0.53 / 2.1 / 10.7 mg/m³ x 6 h	NOAEL: 2.1 mg/m³ LOAEL: 10.7 mg/m³
97	TiO_2 5-6 nm diameter (in-house synthesis)	Mouse CD-1 (ICR) 20 female	Intratracheal instillation 90 days	0 / 2.5 / 5 / 10 mg/kg bw/day	NOAEL: not determined LOAEL: 2.5 kg/kg bw/day

20. For exposure by oral administration, major differences in susceptibility between rats and mice were apparent. Potential intraspecies variability was observed in rats: following exposure over a period of 14 days, a NOAEL and LOAEL at doses of 160 and 400 mg/kg bw/day were determined in Wistar rats, respectively. Sprague-Dawley rats showed no signs of toxicologically relevant effects at doses of 1000 mg/kg bw/day after 90 days of exposure. Nevertheless, mice seemed to be more vulnerable than rats following oral administration of TiO_2 nanomaterials. In ICR (CD-1) mice, toxic effects on several endpoints in spleen have been reported at a dose of 2.5 mg/kg bw/day after 90 days of exposure. Compared with the LOAEL observed in rats, this would point to an interspecies variability factor of 160. However, as studies in rats and mice were not conducted with identical nanomaterial and comparable experimental design, a definitive statement on the interspecies variability factor cannot be given.

Study No.	Material, Description	Species, Strain, Sex and No. per dose	Exposure route, Duration, (Postexposure)	Doses	NOAEL / LOAEL
98	TiO_2 (Sukgyung)	Rat SD 10m + 10f	Oral (gavage) 90 days	0 / 250 / 500 / 1000 mg/kg bw/day	NOAEL: 1000 mg/kgbw/day LOAEL: not determined
99	TiO_2 <50 nm diameter (Sigma)	Rats Wistar 8m + 8f	Oral (gavage) 14 days	0 / 160 / 400 / 1000 mg/kg bw/day	NOAEL: 160 mg/kg bw/day LOAEL: 400 mg/kg bw/day
100	TiO_2 6.5 nm diameter (in-house synthesis)	Mouse ICR (CD-1) 20 female	Oral (gavage) 90 days	0 / 2.5 / 5 / 10 mg/kg bw/day	NOAEL: not determined LOAEL: 2.5 mg/kg bw/day
101	TiO_2 6.9 nm diameter (Hangzhou)	Mouse CD-1 (ICR) 20 female	Oral (gavage) 60 days	0 / 5 / 10 / 50 mg/kg bw/day	NOEL: 5 mg/kg bw/day LOEL: 10 mg/kg bw/day

21. For TiO_2 nanomaterials, various studies with dermal application to rats, mice and pigs were available. However, no statement can be given on interspecies variability as dose-response could not be described adequately (no NOAEL with one exception).

Study No.	Material, Description	Species, Strain, Sex and No. per dose	Exposure route, Duration, (Postexposure)	Doses	NOAEL / LOAEL
102	TiO_2 (Ishihara)	Rat Hairless Wistar Yagi 7 male	Dermal 56 days	0 / 6 mg/cm²	NOAEL: not determined LOAEL: 6 mg/cm²
103	TiO_2 (Ishihara)	Rat Hairless Wistar Yagi 7 male	Dermal 28 days	0 / 6 mg/cm²	NOAEL: not determined LOAEL: 6 mg/cm²
104	TiO_2 (Ishihara)	Rat Hairless Wistar Yagi 7 male	Dermal 14 days	0 / 6 mg/cm²	NOAEL: not determined LOAEL: 6 mg/cm²

Study No.	Material, Description	Species, Strain, Sex and No. per dose	Exposure route, Duration, (Postexposure)	Doses	NOAEL / LOAEL
105	TiO_2 10 nm diameter (Zhejiang)	Mouse BALB/c nu/nu, Grade II 6m + 6f	Dermal 60 days	0 / 0.4 mg/cm² TiO_2	NOAEL: not determined LOAEL: 0.4 mg/cm²
106	TiO_2 25 nm diameter (Zhejiang)	Mouse BALB/c nu/nu, Grade II 6m + 6f	Dermal 60 days	0 / 0.4 mg/cm² TiO_2	NOAEL: not determined LOAEL: 0.4 mg/cm²
107	TiO_2 P25 (Degussa)	Mouse BALB/c nu/nu, Grade II 6m + 6f	Dermal 60 days	0 / 0.4 mg/cm² TiO_2	NOAEL: not determined LOAEL: 0.4 mg/cm²
108	TiO_2 60 nm diameter (Zhejiang)	Mouse BALB/c nu/nu, Grade II 6m + 6f	Dermal 60 days	0 / 0.4 mg/cm² TiO_2	NOAEL: not determined LOAEL: 0.4 mg/cm²
109	TiO_2 4 nm diameter (Zhejiang)	Pig 3 male	Dermal 30 days	Not stated (one dose group)	Not evaluable
110	TiO_2 60 nm diameter (Zhejiang)	Pig 3 male	Dermal 30 days	Not stated (one dose group)	Not evaluable
111	TiO_2 P25 (Degussa)	Pig Yucatan minipigs 3 female	Dermal 22 days	0 / 0.12 mg/cm² TiO_2	NOAEL: 0.12 mg/cm² LOAEL: not determined

22. Overall, indications for interspecies variability exceeding the default assumptions were noted for silicon dioxide, silver, multi-walled carbon nanotubes and titanium dioxide. An analysis for the other nanomaterials evaluated in this report could not be made. Interspecies variability for the substances silicon dioxide, silver and multi-walled carbon nanotubes cannot be confirmed mainly because the studies were conducted with different nanomaterials. A remarkable interspecies variation was noted for titanium dioxide with exposure by oral administration with a 160-fold difference in reported LOAELs. However, confirmation of this finding would be required before a final conclusion can be drawn as different materials were used in rats and mice.

3. Prediction of Deposition and Clearance of Nanomaterials in Rat and Human Lung

23. Considering the lack of relevant human data, it was analysed whether modelling of deposition and retention of nanosilver, –gold and –titanium dioxide in the lung using MPPD (version 2.1) could be used to predict interspecies differences between rat and man. For a description of MPPD, please refer to http://www.ara.com/products/mppd.htm and RIVM, 2002. The following questions were addressed:

- Are there general differences in nanomaterial deposition in rat and human lung?
- Does the modelled lung burden agree with experimentally obtained values?
- What is the relationship between lung burden in rat and humans?

24. MPPD generally predicted higher degrees of tracheobronchial deposition for the studied nanomaterials in humans than in rats, and conversely, a larger fraction of material with alveolar deposition in rats than in humans. Figure 1 shows modelled deposition in rat lung and in human lung for TiO_2, Ag and Au. Irrespective of the type of nanomaterial, the deposited dose per area in the alveolar region was predicted to differ by a factor of approximately 2 at identical particle concentration in the air.

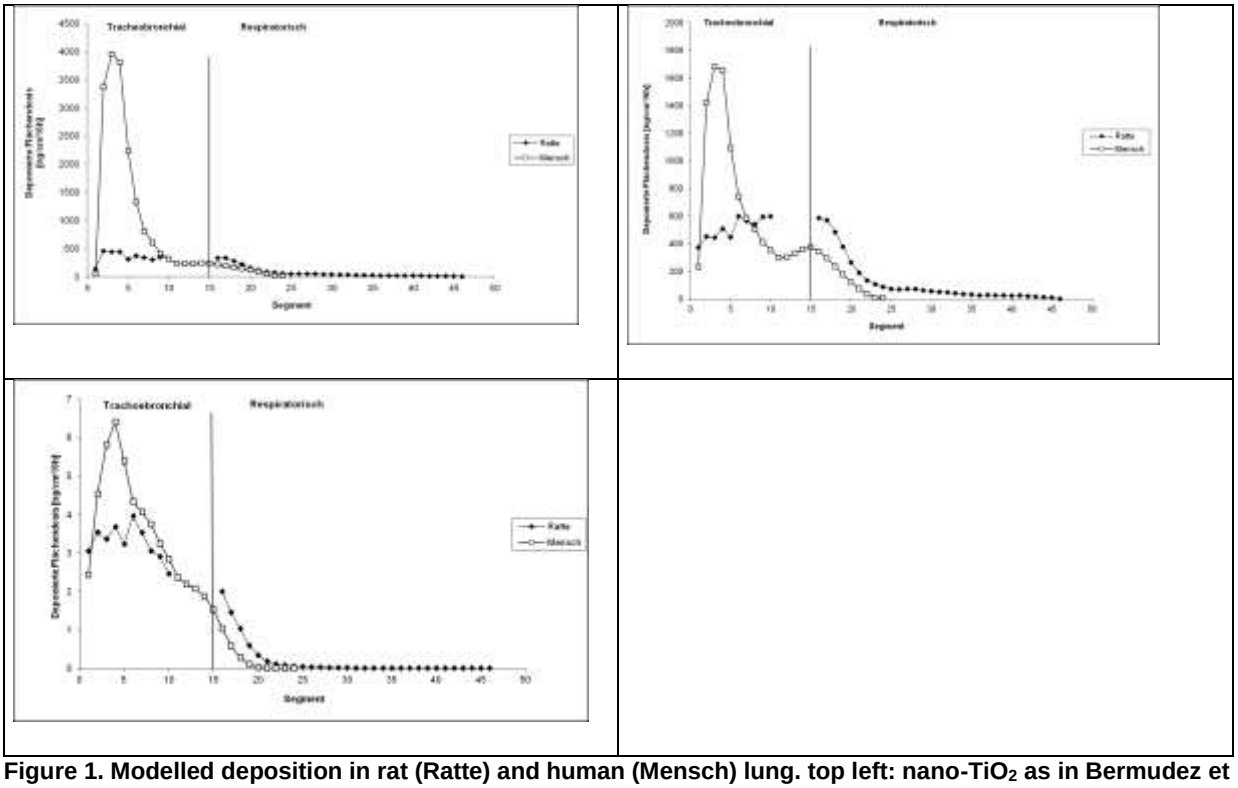

Figure 1. Modelled deposition in rat (Ratte) and human (Mensch) lung. top left: nano-TiO_2 as in Bermudez et al., Toxicol Sci, 2004.; top right: nano-Ag as in Sung et al., 2009.; bottom left: nano-Au as in Sung et al., 2011.

25. Generally, the lung burden modelled using MPPD v2.1 using the same parameters as in acute and subacute inhalation studies in rats was in agreement to measured data. Quantitative differences did usually not exceed the factor of 3. It is noted that this factor is used in regulatory toxicology to account for intraspecies variability in toxicokinetics. A typical example is presented in table 1 below. However, it must be noted that for nanosilver, major differences were noted during the one week post-exposure period following a single day of exposure. The data presented in table 2 may suggest that while the amount of deposition is correctly predicted, retention / clearance is not, at least not for this preparation of nanosilver.

Table 1: Comparison of measured and modelled alveolar retention (mg Ti) in rat following 5 days inhalation exposure to different doses of ultrafine TiO_2 with and without 16 days recovery (measured data from Ma-Hock et al., 2009).

dose	Ti retention [mg]	5 day exposure	+16 day recovery
2 mg/m³			
	measured	0.119	0.093
	MPPD	0.055	0.040
10 mg/m³			
	measured	0.545	0.400
	MPPD	0.371	0.308
50 mg/m³			
	measured	1.635	1.34
	MPPD	1.905	1.75

Table 2: Comparison of measured and modelled alveolar retention (µg Ag) in rat following a single day (6 hours) of inhalation exposure to 0.133 mg/m^3 nanosilver with 0, 1, 4 and 7 days recovery (measured data from Takenaka et al., 2001).

Ag retention [µg]	post-exposure			
	0 days	**1 day**	**4 days**	**7 days**
measured	1.716	0.656	0.152	0.075
MPPD	2.96	2.88	2.6	2.4

26. Importantly, however, there were very significant (>10-fold) and inconsistent deviations for subchronic exposure scenarios in organ burden when comparing modelled and measured data. Examples for nanosilver and nanogold are provided in tables 3 and 4. Findings may, presumably, be explained by deficiencies of the model with respect to nanoparticle lung clearance as a result of solubility or differences in regional deposition and clearance between model and experiment. Therefore, it is also concluded that a refinement of extrapolation of (sub) chronic toxicity data from rats to humans using MPPD by, for example estimation of a Human Equivalent Dose (HEC), requires further model development.

Table 3: Comparison of measured and modelled alveolar retention (µg Ag) in rat following 90 days inhalation exposure to different doses of nanosilver with and without recovery (measured data from Sung et al., 2009)

Ag-retention [µg]			
	end of exposure	**4 weeks recovery**	**12 weeks recovery**
	0.049 mg/m³		
measured	0.564		
MPPD	32		
	0.133 mg/m³ (NOAEC)		
measured	6.01		
MPPD	100		
	0.515 mg/m³		
measured	23.25		
MPPD	478		
	0.117 mg/m³		
measured	0.675	0.361	0.314
MPPD	79	51	21

Table 4: Comparison of measured and modelled alveolar retention (µg Au) in rat following 90 days inhalation exposure to different doses of nanogold (measured data from Sung et al., 2011)

Au-retention [µg]	
	0.00004 mg/m³
measured	0.022837
MPPD	0.001539
	0.0038 mg/m³ (NOAEC)
measured	0.043896
MPPD	0.01498
	0.02002 mg/m³
measured	2.777345
MPPD	0.8882

RECOMMENDATIONS on the Use of Assessment Factors for Intra- and Interspecies Differences in Human Health Risk Assessment of Nanomaterials

27. When applying standard assessment factors (AF) of 10 for intraspecies and 10 for interspecies differences in the human health risk characterisation of nanomaterials, it should be acknowledged that validation of these values has not yet been possible. Lack of validation of AFs for nanomaterials[5] adds further uncertainty to the assessment.

28. Accordingly, the full database available for the particular nanomaterial, including information on materials that may be regarded as similar based on physico-chemical or toxicological considerations should be taken into account. The extended database may allow to improve the understanding of intra- and interspecies differences in the response to the nanomaterial variability, thus reducing the uncertainty of the assessment.

29. When using physiologically based models such as MPPD for assessment of nanomaterials, these should be validated against reliable experimental data obtained with representative nanomaterials. In particular, while the utility of MPPD v2.1 to predict deposition of a nanomaterial in the rat lung was confirmed, its application to modelling of retention and clearance should currently be questioned and requires special attention. This applies in particular to subchronic and chronic settings.

ADDITIONAL RECOMMENDATIONS

Recommendations for further work

30. A general lack of availability of data from repeated-dose toxicity studies in different species was noted in the Expert Opinion prepared in support of the project. In particular, studies of extended duration such as 90-day subchronic or chronic toxicity studies were only available for a minor part of the analysed nanomaterials and routes of exposures. In addition, the majority of the compiled studies did not determine a NOAEL nor a LOAEL. Only few studies determined both. Thus, further testing should be considered on a set of representative materials, with identical materials tested under comparable exposure conditions for various exposure times in different species.

31. Physiologically based models are receiving increased attention in human health risk assessment. With the available data on lung burden following inhalation exposure to nanomaterials, a useful comparison of measured vs. predicted data has been possible in this project for rats, suggesting that further refinement of the MPPD model is required before it can be applied to (sub)chronic scenarios. Unfortunately, corresponding information has not been available for humans, preventing comparisons between rats and humans.

[5] It was noted that uncertainties associated with the use of standard AFs for non-nanomaterials that are remaining despite retrospective validation work should also be acknowledged.

Recommendations with regard to the format of WPMN Dossiers

32. Suggestions on how the utility of WPMN dossiers may be improved were communicated to the WPMN Steering Group on Testing and Assessment (SG-TA) and are, with the publication of those, no longer part of this report.

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