

Guidance on information requirements and chemical safety assessment

Appendix R8-15 Recommendations for nanomaterials applicable to Chapter R.8 Characterisation of dose [concentration] - response for human health



LEGAL NOTICE

This document contains guidance on REACH explaining the REACH obligations and how to fulfil them. However, users are reminded that the text of the REACH regulation is the only authentic legal reference and that the information in this document does not constitute legal advice. The European Chemicals Agency does not accept any liability with regard to the contents of this document.

Guidance on information requirements and chemical safety assessment

Appendix R8-15 Recommendations for nanomaterials applicable to Chapter R.8 Characterisation of dose [concentration] - response for human health

Reference: ECHA-12-G-09-EN

Publ.date: May 2012

Language: EN

© European Chemicals Agency, 2012

Cover page © European Chemicals Agency

Reproduction is authorised provided the source is fully acknowledged in the form "Source: European Chemicals Agency, <http://echa.europa.eu/>", and provided written notification is given to the ECHA Communication Unit (publications@echa.europa.eu).

If you have questions or comments in relation to this document please send them (indicating the document reference, issue date, chapter and/or page of the document to which your comment refers) using the Guidance feedback form. The feedback form can be accessed via the ECHA Guidance website or directly via the following link:

https://comments.echa.europa.eu/comments_cms/FeedbackGuidance.aspx

European Chemicals Agency

Mailing address: P.O. Box 400, FI-00121 Helsinki, Finland

Visiting address: Annankatu 18, Helsinki, Finland

Appendix R8-15: Recommendations for nanomaterials

1. INTRODUCTION

This appendix has been developed in order to provide advice to registrants preparing their registration dossiers for nanomaterials. The content of the appendix implements the advice provided by the REACH Implementation Project on Nanomaterials 3 (RIP-oN 3) on exposure assessment and risk characterization.

The final report of the project contains a large amount of information including applicability of the methods, research gaps etc. This appendix implements only the agreed outputs (i.e. the recommendations for guidance update).

For further information (e.g. research & development requirements or reasoning behind the advice provided for guidance, the reader can refer to the final report of RIP-oN3. (<http://ec.europa.eu/environment/chemicals/nanotech/index.htm>).

2. RECOMMENDATIONS ON CHARACTERISATION OF DOSE/CONCENTRATION-RESPONSE FOR HUMAN HEALTH ARISING FROM RIP-oN 3 for NANOMATERIALS

The parent guidance provides advice to enable the generation of no-effect levels for human health based upon the integration of all available hazard data generated. The approach for generation of derived no effect levels (DNEL(s)) or derived minimum effect levels (DMEL(s)) considers the following steps:

STEP 1: Gather typical dose descriptors and/ or other information on potency

STEP 2: Decide on mode of action (threshold or non-threshold and which next steps(s) to choose

STEP 3: Derivation of effect levels [(DNEL (step 3-1) or DMEL (step 3-2)] or the use of a qualitative approach (step 3-3).

STEP 4: Select the leading health effect

Due to the complexity of the parent guidance, this Appendix has been structured considering the 4 steps, even when no change was applicable, to allow the user a clear view of each step within the process.

2.1 General remarks

2.1.1 Metrics

Section R.8.1.2 summarizes the aspects to be taken into account when deriving a DNEL/DMEL, one of those aspects is the units used (see Section R.8.1.2.7). Specifically, Table R. 8-1 (DN(M)ELs that may need to be derived, and examples on the nomenclature), gives as footnotes the relevant units to be used depending on the case. Regarding nanomaterials it should be noted that metrics such as surface area concentration (cm^2/m^3) and number concentration (number/m^3) might also be relevant. Taking the former into account and also the fact that dust properties change under different conditions, the footnotes 1 and 2 to Table R. 8-1 should be modified as follows:

1 Units for systemic exposure are mg/m^3 , cm^2/m^3 (relevant for nanomaterials) and nanoparticle number/m^3 (especially relevant for fibres) for inhalation, and mg/kg bw for oral and dermal exposure. Other metrics may also be used if this is scientifically justified and a comparable exposure metric is available to enable a risk characterisation ratio to be derived. In addition, when expressing metric information it should be stated on what the size distribution is based e.g. as-produced, as-exposed or as-interacted.

2 Units for local effects are mg/m^3 , cm^2/m^3 (relevant for nanomaterials) and number/m^3 (especially relevant for fibres) for inhalation; and for dermal exposure: mg/cm^2 , $\text{mg}/\text{person}/\text{day}$. Other metrics may also be used if this is scientifically justified and a comparable exposure metric is available to enable a risk characterisation ratio to be derived. In addition, when expressing metric information it should be stated on what the size distribution is based e.g. as-produced, as-exposed or as-interacted.

2.1.2 STEP 1: Gather typical dose descriptors and/ or other information on potency

No specific recommendations have been made for this Step, either because the content is considered equally applicable for nanomaterials or no new information for nanomaterials is available or the text is generic and the recommendation are included in other parts of the documents.

2.1.3 STEP 2: Decide on mode of action (threshold or non-threshold) and which next step(s) to choose

2.1.3.1 Mode of action (threshold/non-threshold)

Section 8.3 covers the second step of the dose-response characterisation process: deciding whether the effects caused by the substance have a threshold or not. In this respect it should be noted that substances may exert carcinogenic/ mutagenic effects either via direct mechanisms or via mechanisms secondary to a threshold effect (e.g. threshold induction of chronic inflammation leading to genotoxicity and/ or carcinogenicity). Carcinogenic/mutagenic effects occurring secondary to a threshold stimulus such as inflammation could also be considered threshold in nature and as such a DNEL can be derived (e.g. induction of lung overload in experimental animals exposed to poorly soluble low toxicity (nano)particles leading to chronic inflammation, oxidative stress and culminating in lung tumour formation).

More information on lung overload can be found in Section 3.1.1 of Appendix R7-1 to Chapter R7a of the Guidance on IR & CSA.

2.1.4 STEP 3: Derivation of effect levels (DNEL (step 3-1) or DMEL (step 3-2) or the use of a qualitative approach (step 3-3).

2.1.4.1 Route to route extrapolation

Section R.8.4.2 explains the use modification of dose descriptors when necessary. This includes the route to route extrapolation, when no relevant data are available for the relevant route of exposure, while Appendix R.8-2 gives examples and more detailed information on route to route extrapolation.

In this respect it should be taken into account that the use of a route-to-route extrapolation in determining health hazards for nanomaterials may not be considered suitable at this time as the use of this approach has yet to be established for nanomaterials. Therefore the use of route-to-route extrapolation for nanomaterials must be scientifically justified on a case-by-case basis.

2.1.4.2 Assessments factors

Section R.8.4.3.1. deals with the assessment factors used to extrapolate experimental data to human situation. Such as inter- and intra-species variation or exposure duration.

2.1.4.2.1 Interspecies differences

Regarding nanomaterials considering interspecies differences for extrapolation the following should be considered:

In deviating from the default assessment factor during the derivation of a DN(M)EL for (nano)particles, a calculation of the actual lung dose could be performed. However as there are considerable differences in ventilation rates, deposition patterns, and clearance rates between humans and animals, all of these factors should be taken into account.

If performing an extrapolative calculation based upon physiological parameters such as ventilation rates, this should be assessed against other calculations performed in the derivation of a DN(M)EL. This is to address potential for duplication of calculations. For example in the calculation of the inhaled dose rate, a species respiratory volume and duration of exposure is taken into account and as such, a starting point modification for these parameters would not need to be performed.

When considering lung deposition, the aerodynamic diameter [and not the true (stokes) diameter] dictates the fractional deposition of a (nano)particle (see Miller 2000 for further explanation of lung deposition). When calculating the deposited dose, this may also be

performed for the zone within the lung showing signs of adverse effects or particle accumulation (e.g. alveolar region) and this could be supported with histopathological findings.

When considering the clearance rates it should be noted that clearance half times refer to insoluble particles and as such these values should not be used for soluble particles.

Once a calculation of the retained dose within the lung has been made for an experimental animal, this can be normalised to a physiological parameter. Sufficient consideration should be given to the use of alternative physiological parameters to body weight, e.g. lung weight, lung surface area or the surface area of the proximal alveolar region (Donaldson et al. 2008). However the use of alternative parameters should be scientifically justified. In addition the use of additional exposure metrics such as (nano)particle surface area or number concentration (especially for fibres) should be considered when performing analysis which should also be scientifically justified.

2.1.4.2.2 Intraspecies differences

Regarding intraspecies differences, relevant substance-specific information on intraspecies variations should always be used to adjust or substitute the default factors. In the case of (nano)particles, the consideration of lowering the default assessment factor due to perceived sensitivities/ insensitivities within a population must be scientifically justified.

2.1.4.2.3 Differences in duration of the exposure

Section R.8.4.3.1. deals with the assessment factors used to extrapolate experimental data to the human situation. When considering differences between the experimental exposure duration and the exposure for the population and scenario under consideration, Table R.8-5 gives default assessment factors for duration extrapolation. However, it is recommended that if substance specific information is available, it should be used to modify the default upwards or downwards.

When considering the use of a higher factor (for instance, if there is indication of potential accumulation), it should be taken into account that for the case of inhalation of poorly soluble, low toxicity (PSLT) particles, exposure at high doses can lead to accumulation within the alveolar spaces, lung interstitium and lung associated lymph nodes which may result in a further increase in toxicity following long term exposure (Morrow, 1988). For further information see Section 3.1.1. of Appendix R7-1 (Recommendations for nanomaterials) to Chapter R.7a.

2.1.4.2.4 Quality of the whole database

The guidance considers the quality of the whole database to be another parameter to take into account, to cover added uncertainty due to deficiencies of the database. When looking at the quality of the whole database used to calculate a DNEL, an extra assessment factor can be applied to account for deficiencies within the data set - including gaps, inconsistencies between studies, or deficiencies in study design. The application of such extra assessment factors is also applicable to nanomaterials and may be particularly relevant due to the general paucity of information surrounding nanomaterials.

One of the aspects to be considered when assessing the quality of the database is consistency. This requires a critical evaluation of the entire body of available data for consistency and biological plausibility. In addition the availability of chronic data (in particular addressing carcinogenic endpoints), and data addressing absorption, systemic availability and accumulation would be seen as reducing uncertainty.

Additionally, when assessing the consistency and biological plausibility of study data against the wider body of literature for nanomaterials, the use of data on the bulk or other forms of the material in place of nano-specific data must be scientifically justified on a case-by-case-basis and may be associated with additional uncertainty.

2.1.4.3 Deriving the DMEL for a non-threshold carcinogen/ mutagen, without adequate cancer data

Section 8.5.3 discusses possible alternatives when no carcinogenicity data are available, such as read across or use of subchronic studies.

Regarding nanomaterials, the use of a read across approach in addressing data gaps for nanomaterials may not be considered suitable at this time as the use of such approaches for nanomaterials has yet to be established. Therefore the potential use of read-across and other non-testing approaches for nanomaterials in deriving an assessment of hazard for humans must be scientifically justified on a case by case basis.

2.1.5 STEP 4: Select the leading health effect(s)

When considering the critical DN(M)EL the recommendations of (the existing) guidance appear equally suitable for nanomaterials. This also applies to the use of the general limits for dust (i.e. if the DN(M)EL derived is above the general limit for dust, this limit should be used instead of the DN(M)EL).

Note that DNELs derived based on substance specific data can never be adjusted upwards based on the general dust limits and that the dust limits can not be used as a surrogate DNEL when there is no data to set a substance-specific DNEL.

2.1.6 Other issues

2.1.6.1 Acute toxicity

DNEL derivation for acute toxicity is explained in Appendix R.8-8. Figure R. 8.5 (and the explanations following them) which show a strategy tree for setting an acute inhalation toxicity DNEL that includes the possibility of using read across. In this respect, it should be noted that the use of non-testing data such as read-across, grouping or (Q)SAR approaches in addressing data gaps for nanomaterials is very limited at this time. In addition to this the use of such *in-silico* models for nanomaterials has also yet to be established. Therefore the potential use of non-testing approaches for nanomaterials in deriving an assessment of hazard for humans must be scientifically justified on a case by case basis.

2.1.6.2 DNEL derivation when a community/national occupational exposure limit (OEL) is available.

Appendix R.8-13 outlines the approach taken in relation to developing a DNEL in situations where an occupational exposure limit already exists, specifically in the case of an EU indicative occupational exposure limit (IOEL), EU binding occupational exposure limit (BOEL) and a nationally adopted occupational exposure limit.

In the case of nanomaterials, when considering the use of an IOEL as a DNEL, the registrant should consider not only whether the route and duration of exposure are the same, but also whether (particle) physico-chemical characteristics, such as particle size distribution, shape and surface area are also the same. The reason for this is that for nanoparticles alterations in physico-chemical attributes such as size, crystallinity, shape (e.g. spherical or fibrous), and surface functionality/ attributes may impact on the relative toxicity of materials of the same chemical composition. As such, the use of an IOEL in place of a DNEL is only suitable where the material physico-chemical attributes are the same as that of the material in question. In situations where such physicochemical characteristics are not the same and read-across is not

scientifically justified, a DNEL should be generated for the same form/material that reflects the true physico-chemical properties of the substance.

REFERENCES

- CA/59/2008rev1. CASG Nano. 2008, "Nanomaterials in REACH", Brussels.
- Cho WS, Duffin R, Poland CA, Howie SE, MacNee W, Bradley M, Megson IL, Donaldson K. 2010, "Metal oxide nanoparticles induce unique inflammatory footprints in the lung: important implications for nanoparticle testing." *Environ Health Perspect.* ;118(12):1699-706.
- Christensen FM, Johnston HJ, Stone V, Aitken RJ, Hankin S, Peters S, Aschberger K. 2010a "Nano-silver - feasibility and challenges for human health risk assessment based on open literature". *Nanotoxicology*, 4(3):284-95.
- Christensen FM, Johnston HJ, Stone V, Aitken RJ, Hankin S, Peters S, Aschberger K. 2010b "Nano-TiO₂ - feasibility and challenges for human health risk assessment based on open literature". *Nanotoxicology*, Early online, 1-15.
- Donaldson K, Borm PJ, Oberdorster G, Pinkerton KE, Stone V, Tran CL. 2008
"Concordance between in vitro and in vivo dosimetry in the proinflammatory effects of low-toxicity, low-solubility particles: the key role of the proximal alveolar region." *Inhal Toxicol*;20(1):53-62
- Donaldson K. 2009 "The inhalation toxicology of p-aramid fibrils". *Crit Rev Toxicol*;39(6):487-500.
- Duffin, R., Tran, L., Brown, D., Stone V. and Donaldson, K. 2007, "Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: highlighting the role of particle surface area and surface reactivity", *Inhal Toxicol*, vol. 19, no.10, pp. 849-856.
- ENV/JM/MONO(2009)20/REV. OECD. 2010, "Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme; First Revision", Series on the safety of manufactured nanomaterials, No.25, Paris.
- ENV/JM/MONO(2010)25. OECD. 2010, "Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials", Series on the safety of manufactured nanomaterials, No.24, Paris.
- Hanai, S., Kobayashi, N., Ema, M., Ogura, I., Gamo, M., Nakanishi, J. 2009, "Risk assessment of manufactured nanomaterials - titanium dioxide (TiO₂)", Interim Report, Executive Summary, NEDO Project - Research and Development of Nanoparticle Characterization Methods, P06041.
- Hamilton, R.F. Jr., Wi, n., Porter, D., Buford, M., Wolfarth, M., Holian, A. 2009, "Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity", *Part Fibre Toxicol*, vol. 6, no. 36.
- Hsu, L.Y. and Chein, H.M. 2007, "Evaluation of nanoparticle emission for TiO₂ nanopowder coating materials", *Journal of Nanoparticle Research*, vol. 9, no. 1, pp. 157-163.
- Kobayashi, N., Naya, M., Endoh, S., Maru, J., Yamamoto, K. & Nakanishi, J. 2009a, "Comparative pulmonary toxicity study of nano-TiO₂ particles of different sizes and agglomerations in rats: Different short- and long-term postinstillation results", *Toxicology*, vol. 264, no. 1-2, pp. 110-118.
- Kobayashi, N., Ogura, I., Gamo, M., Kishimoto, A., Nakanishi, J. 2009b, "Risk assessment of manufactured nanomaterials - carbon nanotubes (CNTs)", Interim Report, Executive Summary, NEDO Project - Research and Development of Nanoparticle Characterization Methods, P06041.
- Lipka J, Semmler-Behnke M, Sperling RA, Wenk A, Takenaka S, Schleh C, Kissel T, Parak WJ, Kreyling WG. 2010 "Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection." *Biomaterials*. 31(25):6574-81.

Ma-Hock, L., Treumann, S., Strauss, V., Brill, S., Luizi, F., Mertler, M., Wiench, K., Gamer, A.O., van Ravenzwaay, B., Landsiedel, R. 2009, "Inhalation toxicity of multi-walled carbon nanotubes in rats exposed for 3 months," *Toxicol Sci*, vol. 112, pp. 273-275.

Mercer, R.R., Hubbs, A.F., Scabilloni, J.F., Wang, L., Battelli, L.A., Schwegler-Berry, D., Castranova, V., Porter, D.W. 2010, "Distribution and persistence of pleural penetrations by multi-walled carbon nanotubes," *Part Fibre Toxicol.*, vol. 7, pp. 28.

Morrow, P.E. 1988, "Possible mechanisms to explain dust overloading of the lungs," *Fundam. Appl. Toxicol.* vol. 10, pp. 269-384.

Mylon, S.E., Chen, K.L. & Menachem, E. 2004, "Influence of Natural Organic Matter and Ionic Composition on the Kinetics and Structure of Hematite Colloid Aggregation: Implications to Iron Depletion in Estuaries Langmuir," vol. 20, no. 21, pp. 9000-9006.

NIOSH (National Institute for Occupational Safety and Health) 2005. NIOSH Current Intelligence bulletin: Evaluation of Health Hazard and Recommendations for Occupational Exposure to Titanium Dioxide. DRAFT. NIOSH. Accessed at: <http://www.cdc.gov/niosh/review/public/TIO2/pdfs/TIO2Draft.pdf> (4th September 2010)

Oberdörster G. 2010, "Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. *J Intern Med.*; 267(1):89-105.

OECD. 2010, Publications in the Series on the Safety of Manufactured Nanomaterials,

http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1_00.html

(Accessed September 2010).

Oller AR, Oberdörster G. 2010 "Incorporation of particle size differences between animal studies and human workplace aerosols for deriving exposure limit values". *Regul Toxicol Pharmacol.*;57(2-3):181-94.

Pauluhn, J. 2010a, "Multi-walled carbon nanotubes (Baytubes): Approach for derivation of occupational exposure limit," *Regul Toxicol Pharmacol*, vol. 57, pp 78-89.

Pauluhn, J. 2010b, "Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures," *Toxicol Sci*, vol. 113, no. 1, pp. 226-242.

Poland, C., Duffin, R., Kinloch, I., Maynard, A.D., Wallace, W., Seaton, A., Stone.V., Brown, S., MacNee, W., and Donaldson, K. (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology*, vol. 3, 423-428.

RIVM. 2009, "Nanomaterials under REACH: Nanosilver as a case study", RIVM Report 601780003/2009.

RNC/RIP-oN3/A/1/FINAL. Final Report on Task A: Identification and Review of Information Sources

RNC/RIP-oN3/B1/2/FINAL. Final Report on Task B1: Exposure Scenario Case Studies

RNC/RIP-oN3/B2/2/FINAL. Final Report on Task B2: Operational conditions and risk management measures - harvesting results from on-going activities

RNC/RIP-oN3/B3/2/FINAL. Final Report on Task B3: Exposure estimation (modelling and measurements) - harvesting results from on-going activities

RNC/RIP-oN3/B4/2/FINAL. Final report on Task B4: Advisory Report on Operational Conditions, Risk Management Measures, Exposure Scenarios and Exposure Estimation (modelling and measurement) with the Purpose of Conducting Exposure Assessment of Nanomaterials for REACH purposes

RNC/RIP-oN3/C1/2/FINAL. Case-studies on how no-effect-levels for health and the environment could be established

RNC/RIP-oN3/C2/2/FINAL. Final Report on Task C1: Case-studies on how no-effect-levels for health and the environment could be established

RNC/RIP-oN3/C3/2/FINAL. Final Report on Task C3: Advisory Report on Hazard and Risk Characterisation of Nanomaterials

RNC/RIP-oN3/D/2/FINAL. Joint Final Report on RIP-oN2 Task C & RIP-oN3 Task D: Metric(s) to compare in the risk characterisation

Roberts, A.P., Mount, A.S., Seda, B., Souther, J., Qiao, R., Lin, S.J., Ke, P.C., Rao, A.M. and

Klaine, S.J. 2007, "In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*", *Environ.Sci.Technol.*, vol. 41, no. 8,

pp. 3025-3029.

Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, Moss OR, Wong BA, Dodd DE, Andersen ME, Bonner JC. 2009 "Inhaled carbon nanotubes reach the subpleural tissue in mice". *Nat Nanotechnol*;4(11):747-51.

Tran, C.L., Buchanan, D., Cullen, R.T., Searl, A., Jones, A.D., Donaldson, K. 2000, "Inhalation of poorly soluble particles. II. Influence Of particle surface area on inflammation and clearance". *Inhal Toxicol.* vol. 12, no. 12, pp. 1113-

Warheit DB, Webb TR, Reed KL, Frerichs S, Sayes CM. 2007 "Pulmonary toxicity study in rats with three forms of ultrafine-TiO₂ particles: differential responses related to surface properties". *Toxicology.* 25;230(1):90-104.

EUROPEAN CHEMICALS AGENCY
ANNANKATU 18, P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU