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EUROPEAN CHEMICALS AGENCY

GUIDANCE

Guidance on information requirements and chemical safety assessment

Appendix R7-2 Recommendations for nanomaterials applicable to Chapter R7c Endpoint specific guidance

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LEGAL NOTE

Guidance on information requirements and chemical safety assessment
Extracts from Appendix R7-2 Recommendations for nanomaterials applicable to Chapter R7c - Endpoint specific guidance

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NOTE

Please note that the present document is a proposed amendment to specific extracts **only** of *Appendix R7-2 to Chapter R.7c* of the IR&CSA Guidance.

This document was prepared by the ECHA Secretariat for the purpose of this consultation and includes only the parts open for the current consultation, i.e. :

- Section 2.1.1 Aquatic bioaccumulation (section 1.1.1 in this revised version)

- Section 2.1.2 Effects on terrestrial organisms (section 1.1.2 in this revised version)

http://echa.europa.eu/documents/10162/13632/appendix_r7c_nanomaterials_en.pdf (version 1.0 published in April 2012).

The numbering and headings of the sub-sections that are displayed in the document for consultation correspond to those used in the currently published guidance document; this will enable the comparison of the draft revised sub-sections with the current text if necessary.

After conclusion of the consultation and before final publication the updated sub-sections will be implemented in the full documents.

DOCUMENT HISTORY

Version	Changes	Date
Version 1	First edition	April 2012
Version 2	• Update of section 1.1.1. on aquatic bioaccumulation, to explain the general limitations of the K_{ow} as the basis for a waiver for nanomaterials and provide advice on the applicability of the available OECD guidelines; • Update of section 1.1.2 on Effects on terrestrial organisms to provide advice on spiking methods and use of different metrics. Please note that the numbering of the sections has changed, the section numbers above refer to the updated numbering of the guidance	Xxxx 2017

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1 RECOMMENDATIONS FOR ECOTOXICOLOGICAL ENDPOINTS for NANOMATERIALS:

1.1 Specific advice for endpoints

When following the endpoint specific advice provided by this guidance, please take into account that the advice regarding sampling preparation provided in section 2.1.1 of *Appendix R7-1 to ECHA Guidance R.7.a* and the general advice on ecotoxicity and fate testing provided in section 1.1 of *Appendix R7-1 to ECHA Guidance R.7.b* are also applicable for this guidance.

1.1.1 Aquatic bioaccumulation

In the Parent ECHA Guidance, section R.7.10.2 describes the REACH Annex IX information requirements for aquatic bioaccumulation and the use of alternative information when measured data are not available. However, the prediction techniques described in the parent guidance and the use of surrogate information (e.g. the octanol-water partitioning coefficient K_{ow}), applicable for many classes of organic substances, may not be applicable to predict bioaccumulation potential of nanoparticles. In the case of nanomaterials, normally, it is not possible to make log K_{ow} or solubility estimations since nanomaterials are dispersed and not in solution. However, measurement of n-octanol/water partition coefficient may still be of value for organic nanomaterials that are water soluble and have a high dissolution rate.

1.1.1.1 Non-testing data

Section R.7.10.3.2 of the parent guidance concerns non-testing data, e.g. quantitative structure-activity relationships (QSARs), bioconcentration factor (BCF) models based on log K_{ow} and grouping approaches for assessing aquatic bioaccumulation. The use of *in silico* models for nanomaterials has yet to be established or accepted and therefore, when used, needs to be thoroughly reported and justified. With regard to nanoparticles, it is often not possible to make bioaccumulation estimations based on log K_{ow} or solubility, as explained above and in the Appendix R7-1 to ECHA Guidance R.7.a [1] Sections 2.2.1, 2.2.2 and 2.2.4. Nevertheless, non-testing methods and parameters like the ones listed under *Appendix R7-1 to ECHA Guidance R.7.a*, could be useful for this endpoint when considered as part of a weight of evidence approach.

Section R.7.10.3.4 of the parent Guidance describes other indicators for bioaccumulation potential. This includes a screening approach where potential bioaccumulation can be estimated from the value of the n-octanol/water partition coefficient (K_{ow}). Furthermore, REACH Annex IX 9.3.2 column 2 states that, for instance, a value for log $K_{ow} \leq 3$ could be used as a waiving argument to omit the testing of bioaccumulation in aquatic species. This approach is not necessarily appropriate for nanoparticles, as prediction techniques based on equilibrium partitioning do not strictly apply to undissolved nanoparticles- as explained in Appendix R7-1 to ECHA Guidance R.7.a Sections 2.2.1, 2.2.2 and 2.2.4. As outlined in OECD 40 [3], the K_{ow} value is not often suitable for predicting bioaccumulation for nanomaterials.

Taking into account the above, waiving the information requirement for bioaccumulation in aquatic species based on log K_{ow}, log K_{oc} or other screening methods is in most cases not appropriate for nanomaterials.

1.1.1.2 *In vivo* tests for aquatic bioaccumulation

The parent guidance section R.7.10.3.1 describes the OECD TG 305 Bioaccumulation in Fish [4] : Aqueous and Dietary Exposure as an appropriate *in vivo* test method to fulfil the information requirement set for bioaccumulation in aquatic species in Annex IX 9.3.2. Further information on bioaccumulation testing strategy can be found in *Chapter R.11 of the Guidance on IR&CSA on PBT* assessment.

OECD TG 305 is partially applicable for nanomaterials. It is applicable when the dietary exposure route is followed; the aqueous exposure route resulting in a *bioconcentration factor (BCF)* is not applicable for most nanomaterials, if they remain as nanoparticles. For organic nanomaterials that are water soluble and/or would have a high dissolution rate, a BCF study is applicable via the aqueous route. However, there may be a need for additional considerations and testing for bioaccumulation of the particular form of such nanomaterials. BCF is the ratio of the concentration of a substance in an organism to the concentration in water, once a steady state has been achieved. For nanoparticles, a BCF cannot be calculated as no thermodynamic equilibrium will be reached between the organism and the water phase [5] and a stable aqueous concentration cannot be maintained. Nevertheless, uptake and depuration rate as kinetic data can be assessed instead for nanomaterials and particles. Therefore provided these kinetic parameters are used and estimated, the flow through method can still be applied for the nanomaterials bioaccumulation estimation ([3], [6], [7] and [8]).

A new OECD Guidance for assessing the apparent accumulation potential for nanomaterials is under development. This guidance, when available, will provide information on how to test nanomaterials via the dietary exposure and on how to measure and quantify the accumulation potential in fish. In the meantime, the existing draft GD on dietary exposure can give information on that exposure method[1] .

Other *In vivo* tests for bioaccumulation could be also used, apart from the testing in aquatic media, such as bioaccumulation in sediment and soil. OECD TG 315 Bioaccumulation in Sediment dwelling Benthic Oligochaetes [9] and OECD TG 317 Bioaccumulation in Terrestrial Oligochaetes [10] are in principle applicable for nanomaterials, but expert judgement will be required for performing the bioaccumulation tests and interpreting the results ([8], [11])... The results of applying these TGs (OECD TG 315 and OECD TG 317), taking into account the current challenge in testing bioaccumulation of nanomaterials in fish, may be used as weight of evidence in bioaccumulation assessment. Soil and sediment compartments are considered potential sinks for nanomaterials and therefore they are also relevant when considering nanomaterial fate in the environment.

Whenever tests for bioaccumulation in aquatic or sediment and soil organisms are performed, in order to be considered reliable, the recommendations on sample preparation and ecotoxicity and fate testing given in Appendix R7-1 to chapter R7a, section 2.1.1. (Sample preparation) and Appendix R7-1 to R7b, section 2.1 (General advice on how to perform nanomaterials ecotoxicity and fate testing) should be followed. In addition, test concentrations should be monitored throughout the whole test duration to account for concentration-specific changes in dispersion and agglomeration/aggregation characteristics, using mass metric and nano-specific metrics e.g. surface area, particle number, when relevant ([8], [11]).

1.1.2 Effects on terrestrial organisms

1.1.2.1 Non-testing data

In the parent guidance R7c, and also part a) of Section R.7.11.3.1, the possibility of using non-testing approaches e.g. QSAR, grouping and the equilibrium partitioning method (EPM) to estimate soil and terrestrial toxicity is explained.

Regarding nanomaterials, estimates based on "partitioning" are limited to distribution of a substance in molecular form. In the case of nanoparticles, the partitioning method may underestimate exposure in soil and sediment environments and overestimate the exposure in water. If the particle size is small, distribution via air may also occur. There are no estimation methods available for particle distribution, so this has to be dealt with on a case-by-case basis.

[1] Available at: http://www.oecd.org/env/ehs/testing/draft-guidance-review-documents-monographs.htm

1.1.2.2 Testing data

Regarding testing on effects on terrestrial organisms, the methods described in the parent guidance Section R.7.11 are in principle applicable for testing nanomaterials.
The application technique in e.g. sample preparation and spiking has been shown to have an effect on the availability of the nanomaterial and its level of ecotoxicity in soil [6]. Therefore it is essential that the sample preparation and spiking method applied is well justified and reported in detail, and that the recommendations set in the OECD Guidance Manual for the Testing Manufactured Nanomaterials: OECD's Sponsorship Programme; first revision [12] (OECD, 2009), Guidance Notes on Sample Preparation and Dosimetry for nanomaterials [13] and OECD 40 [3] are followed.

When performing the test, the test material needs to be homogenously dispersed in the soil. OECD 40 [3] describes different spiking methods; particles can be dispersed as aquatic dispersion into soil (wet spiking) or directly into test media (dry spiking), or put onto a carrier e.g. silica sand or spiked food. The optimal spiking method depends on both the test material and the test method. It will depend on the physicochemical properties of the nanomaterial, the target concentration, the medium, and the bioassay method selected, and preliminary data gathered prior to the test. For example, ZnO nanoparticles can be introduced to soil as aqueous solutions prepared in the soil extracts to achieve homogenous distribution [14] and satisfactory spiking homogeneity can be achieved with Ag nanoparticles using soil as a solid carrier [6].

Unless the use of mass metric only can be justified, nano-specific metrics such as particle number and surface area should in principle be used when relevant. Using multiple metrics allows retrospective correlation of the measured response with different dose metrics, (see Section 2.1.1 of Appendix to Chapter R7.b). If e.g. only mass metric is recorded during the test, conversion between metrics increases the uncertainty in interpretation of the test results and therefore measurement of multiple metrics during testing is recommended (as highlighted in section 2.1.1 of *Appendix R7-1 to ECHA Guidance R.7.a)*.

In addition to these recommendations, it should be considered that measurements of the nanomaterial's concentration (using different metrics, e.g. particle number, surface area, or mass concentration) should be monitored throughout the test at all test concentrations to account for concentration-specific changes in dispersion and agglomeration/aggregation characteristics if possible ([9], [11].

1
2 # REFERENCES

3

[1] ECHA, "Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance," [Online]. Available: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

[2] ECHA, "[DRAFT] Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance on QSARs and Grouping," [Online]. Available: http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach.

[3] OECD, "Series on the Safety of Manufactured Nanomaterials- No. 40. Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. Expert Meeting Report ENV/JM/MONO(2014)1," 2014. [Online]. Available: http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm.

[4] OECD, "Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure, OECD Guidelines for the Testing of Chemicals, Section 3," 2012. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x.

[5] G. Cornelis, "Fate descriptors for engineered nanoparticles: the good, the bad, and the ugly," *Environmental Science: Nano,* vol. 2, pp. 19-26, 2015.

[6] K. Hund-Rinke, K. Schlich and T. Klawonn, "Influence of application techniques on the ecotoxicological effects of nanomaterials in soil," *Environmental Sciences Europe,* vol. 24, no. 1, pp. 1-12, 2012.

[7] L. M. Skjolding, *Bioaccumulation and trophic transfer of engineered nanoparticles in aquatic organisms-PhD thesis DTU, Denmark,* 2015.

[8] K. Rasmussen, M. Gonzalez, P. Kearns, J. Riego Sintes, F. Rossi and P. Sayre, "Review of achievements of the OECD Working Party on Manufactured Nanomaterials' Testing and Assessment Programme. From exploratory testing to test guidelines," *Regulatory Toxicology and Pharmacology,* vol. 74, p. 147–160, 2016.

[9] OECD, "Test No. 315: Bioaccumulation in Sediment-dwelling Benthic Oligochaetes, OECD Guidelines for the Testing of Chemicals, Section 3," 2008. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x.

[10] OECD, "Test No. 317: Bioaccumulation in Terrestrial Oligochaetes, OECD Guidelines for the Testing of Chemicals, Section 3," 2010. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-3-degradation-and-accumulation_2074577x.

[11] K. Hund-Rinke, M. Herrchen and K. Schlich, "Integrative test strategy for the environmental assessment of nanomaterials. UBA-FB 00," 2014. [Online]. Available: http://www.bmub.bund.de/fileadmin/Daten_BMU/Pools/Forschungsdatenbank/fkz_3712_65_409_nanomaterialien_teststrategie_bf.pdf.

[12] OECD, "Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme. First revision", Series on the safety of manufactured nanomaterials, ENV/JM/MONO(2009)20/REV," 2009. [Online]. Available: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)20/rev&doclanguage=en.

[13] OECD, "Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterial. Series on the Safety of Manufactured Nanomaterials No. 36. ENV/JM/MONO(2012)40," 2012. [Online]. Available: http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm.

[14] P. Kool, M. Ortiz and C. van Gestel, "Chronic toxicity of ZnO nanoparticles, non-nano

ZnO and ZnCl2 to Folsomia candida (Collembola) in relation to bioavailability in soil," *Environ Pollut.,* vol. 159, no. 10, pp. 2713-2719, 2011.

[15] E. J. Petersen, S. A. Diamond, A. Kennedy, G. Goss, K. Ho, J. Lead, S. Hanna, N. Hartmann, K. Hund-Rinke, B. Mader, N. Manier, P. Pandard, E. Salinas and P. Sayre, "Adapting OECD Aquatic Toxicity Tests for Use with Manufactured Nanomaterials: Key Issues and Consensus Recommendations," *Environmental Science & Technology,* vol. 49, no. 16, p. 9532–9547, 2015.

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