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GUIDANCE

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

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

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Version 1	First edition	April 2012
Version 2.0	• Update of section 1.1.1. on aquatic bioaccumulation, to explain the general limitations of K_{ow} as a basis for a waiver for nanomaterials and to 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 on use of different metrics. Please note that the numbering of the sections and sub-sections has changed from that in Version 1, the section numbers above refer to the updated numbering of the guidance as used in Version 2.0 (onwards).	May 2017

PREFACE

Three appendices concerning information requirements (appendices to IR&CSA Guidance Chapters R7a, R7b and R7c) have been developed in order to provide advice to registrants for use when preparing REACH registration dossiers that cover "nanoforms"[1].

The advice provided in this document focuses on specific recommendations for testing materials that are nanomaterials[2]. Part of the advice provided is not strictly nano-specific and may for instance also be applicable to other particulate materials (e.g. relevance of dissolution rate). However, when such advice has been included, it is because it is considered that the issue covered is especially relevant for nanomaterials and should be part of the nano-specific guidance.

In the absence of availability of any suitable specific provision (either because the endpoint is not relevant for nanomaterials, because the guidance already provided is considered to be equally applicable to nanomaterials as to non-nanomaterials, or because more research is needed before developing advice) no additional guidance for the endpoint has been included in this appendix.

This appendix intends to provide advice specific to nanomaterials and does not preclude the applicability of the general principles given in Chapter R.7c (i.e. the parent guidance). Moreover, when no advice has been given in this appendix for a specific endpoint the advice provided in the parent Guidance should be followed.

Please note that this document (and its parent guidance) provides specific guidance on meeting the information requirements set out in Annexes VI to XI to the REACH Regulation.

General information for meeting the information requirements such as collection and evaluation of available information, and adaptation of information requirements is available in Chapter R.2 to R.5 of Guidance on IR&CSA).

Moreover, when considering the use of data already available *Appendix R.6-1: for nanomaterials applicable to the Guidance on QSARs and Grouping* of *Chemicals* [1] may be useful as it provides an approach on how to justify the use of hazard data between nanoforms (and the non-nanoform) of the same substance.

[1] Please see *How to prepare registration dossiers that cover nanoforms: best practices* [21]
[2] See Recommendation on the definition of nanomaterial adopted by the European Commission

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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 sample 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 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 partition 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, it is not normally 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 Appendix R7-1 to ECHA Guidance R.7.a [2] Sections 2.2.1, 2.2.2 and 2.2.4. Nevertheless, non-testing methods and parameters such as those listed in *Appendix R7-1* to the ECHA IR&CSA Guidance chapter 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 justify omitting 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 chapter R.7a of the ECHA IR&CSA guidance Sections 2.2.1, 2.2.2 and 2.2.4. As outlined in OECD 40 [3], the K_{ow} value is often not 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 OECD TG 305 "Bioaccumulation in Fish: Aqueous and Dietary Exposure" [4] 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 strategies can be found in *Chapter R.11 of the Guidance on IR&CSA*, concerning 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. The BCF is the ratio of the concentration of a substance in an organism to its 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 estimation of the nanomaterial's bioaccumulation potential ([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[3] .

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.

In order for them to be considered reliable, whenever tests for bioaccumulation in aquatic or sediment and soil organisms are performed, 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 a mass metric and nano-specific metrics such as 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 (Chapter R7c), 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.

With respect to nanomaterials, estimates based on "partitioning" are limited to distribution of a substance in molecular form (excluding ionic forms as explained in parent guidance). 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

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

distribution, so this has to be dealt with on a case-by-case basis.

1.1.2.2 Testing data

Regarding testing for effects on terrestrial organisms, the methods described in the parent guidance Section R.7.11 are, in principle, also 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 are well justified and reported in detail, and that the recommendations set out in the OECD Guidance manual for the testing of 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 dispersions 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 the mass metric only can be justified, nano-specific metrics such as particle number and surface area should in principle be used whenever 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 the 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].

Appendix R7-2 to Chapter R.7c

2.1.3 Guidance on Toxicokinetics

A toxicokinetics study is not an information requirement under REACH. However, as for all other substances, the standard information requirements defined by the REACH regulation can give useful information to help make a judgement about the possible toxicokinetics of nanomaterials (See Section R.7.12.2.1).

Information on the possible behaviour of the nanomaterials can be supplemented with *in vitro* and *in silico* predictions based on physicochemical and other data. This information may be used in grouping of nanomaterials to assist in the read-across of exposure and hazard characteristics, thereby reducing the total number of tests required.

It is acknowledged that nanomaterials' properties may alter the ADME (absorption, distribution, metabolism, and excretion) behaviour in comparison to non-nano-sized forms. The toxicokinetic profile of nanomaterials may depend on several physicochemical parameters, e.g. composition, size, shape, surface area, agglomeration/aggregation state, surface properties (including surface charge), hydrophobicity, and dissolution. Therefore, nanomaterials may be able to reach unexpected parts of the body that are otherwise protected from exposure to particulate materials by biological barriers. It is noted that detecting and quantifying nanoparticles in biological tissue is still analytically and technically challenging. Therefore, it is recommended that the methods used and their limitations be adequately documented.

Data on solubility and dissolution rate in relevant biological fluids and testing media is an essential starting point in understanding a particle's behaviour and ADME properties and to set boundaries for considering a substance as "poorly soluble" (See Section 3.1.1 of *Appendix R.7-1 for nanomaterials applicable to the Chapter R.7a*.). Determination of the dissolution rate provides an insight into how a specific particle may interact with its biological environment [15].

In the case of PSPs, it is of paramount importance to determine whether or not they may cross biological barriers. Translocation may be further influenced by the properties listed in Section 3.1 of *Appendix R.7-1 for nanomaterials applicable to the Chapter R.7a*.

In addition to hazard assessment, the information on toxicokinetics is valuable to justify the use of toxicological data between different forms of a substance (*Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping* [1]). Therefore, in order to optimise animal use it is highly recommended to collect as much toxicokinetics data as possible from the experiments required under REACH. For example, when dose range finding studies or main repeated dose, reproductive or genotoxicity studies are performed, for poorly soluble nanomaterials, several additional analyses could be considered such as:

- Urine and faeces sampling
- Microscopic or electron microscopic qualitative determination of the presence of nanomaterials in the relevant tissues when (technically) feasible. Alternatively, other methods such as multiplexed imaging by use of laser desorption/ionization mass spectrometry LDI-MS, Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) etc could be used ([16], [17]).
- Sampling at several time points in different organs to monitor the fate and accumulation of the particles in the body (data from range-finding studies could be used to determine the appropriate sampling times)
- Lung and tissue burden

It could be useful to keep the samples to allow later analysis. (E.g. storage by freezing or tissue fixation for microscopy ([18]), freezing for burden analysis ([19], [20])). It is not intended here to advise on use of extra animals for the additional analyses unless scientifically justified. However, it is important to balance between performing additional analyses and indication of toxicity

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