

GUIDANCE

Guidance on information requirements and chemical safety  
assessment

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

Draft (Public) Version 2.0

December 2016



## LEGAL NOTE

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### **Guidance on information requirements and chemical safety assessment**

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

**Reference:** ECHA-XXXXXX-EN

**ISBN:** XXXXXX

**Publ.date:** XXXXXX

**Language:** EN

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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 2.1.2 Effects on terrestrial organisms

[http://echa.europa.eu/documents/10162/13632/appendix\\_r7c\\_nanomaterials\\_en.pdf](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.

1 **DOCUMENT HISTORY**

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Version	Changes	Date
Version 1	First edition	April 2012
Version 2	<ul style="list-style-type: none"><li>Update of section 2.1.1. on aquatic bioaccumulation, to explain the general limitations of the <math>K_{ow}</math> as the basis for a waiver for nanomaterials and provide advice on the applicability of the available OECD guidelines;</li><li>Update of section 2.1.2 on Effects on terrestrial organisms to provide advice on spiking methods and use of different metrics.</li></ul>	Xxxx 2017

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## 2 RECOMMENDATIONS FOR ECOTOXICOLOGICAL ENDPOINTS for NANOMATERIALS:

### 2.1 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.

#### 2.1.1 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.

##### 2.1.1.1 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 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.

##### 2.1.1.2 *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.

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

14  
15 A new OECD Guidance for assessing the apparent accumulation potential for nanomaterials is  
16 under development. This guidance, when available, will provide information on how to test  
17 nanomaterials via the dietary exposure and on how to measure and quantify the accumulation  
18 potential in fish. In the meantime, the existing draft GD on dietary exposure can give  
19 information on that exposure method<sup>1</sup>.

20  
21 Other *In vivo* tests for bioaccumulation could be also used, apart from the testing in aquatic  
22 media, such as bioaccumulation in sediment and soil. OECD TG 315 Bioaccumulation in  
23 Sediment dwelling Benthic Oligochaetes [9] and OECD TG 317 Bioaccumulation in Terrestrial  
24 Oligochaetes [10] are in principle applicable for nanomaterials, but expert judgement will be  
25 required for performing the bioaccumulation tests and interpreting the results ( [8], [11])...  
26 The results of applying these TGs (OECD TG 315 and OECD TG 317), taking into account the  
27 current challenge in testing bioaccumulation of nanomaterials in fish, may be used as weight of  
28 evidence in bioaccumulation assessment. Soil and sediment compartments are considered  
29 potential sinks for nanomaterials and therefore they are also relevant when considering  
30 nanomaterial fate in the environment.

31  
32 Whenever tests for bioaccumulation in aquatic or sediment and soil organisms are performed,  
33 in order to be considered reliable, the recommendations on sample preparation and ecotoxicity  
34 and fate testing given in Appendix R7-1 to chapter R7a, section 2.1.1. (Sample preparation)  
35 and Appendix R7-1 to R7b, section 2.1 (General advice on how to perform nanomaterials  
36 ecotoxicity and fate testing) should be followed. In addition, test concentrations should be  
37 monitored throughout the whole test duration to account for concentration-specific changes in  
38 dispersion and agglomeration/aggregation characteristics, using mass metric and nano-specific  
39 metrics e.g. surface area, particle number, when relevant ( [8] , [11]).

## 41 **2.1.2 Effects on terrestrial organisms**

### 42 **2.1.2.1 Non-testing data**

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

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

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

1

2 Testing data

3 Regarding testing on effects on terrestrial organisms, the methods described in the parent  
4 guidance Section R.7.11 are in principle applicable for testing nanomaterials.

5 The application technique in e.g. sample preparation and spiking has been shown to have an  
6 effect on the availability of the nanomaterial and its level of ecotoxicity in soil [6]. Therefore it  
7 is essential that the sample preparation and spiking method applied is well justified and  
8 reported in detail, and that the recommendations set in the OECD Guidance Manual for the  
9 Testing Manufactured Nanomaterials: OECD's Sponsorship Programme; first revision [12]  
10 (OECD, 2009), Guidance Notes on Sample Preparation and Dosimetry for nanomaterials [13]  
11 and OECD 40 [3] are followed.

12

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

23

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

31

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

37



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