

GUIDANCE

Guidance on information requirements and chemical safety
assessment

**Appendix R7-1 Recommendations for nanomaterials
applicable to Chapter R7a Endpoint specific guidance**

Draft (Public) Version 2.0

December 2016



NOTE

Please note that the present document is a proposed amendment to specific extracts **only** of the *Appendix R7-1 to Chapter R.7a* of 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 on sample preparation

- Section 2.2.1 Water solubility

- Section 2.2.2 Partition coefficient n-octanol/water

- Section 2.2.4 Adsorption/desorption

The full guidance document (version before proposed amendments) is available on the ECHA website at:

http://echa.europa.eu/documents/10162/13632/appendix_r7a_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 **LEGAL NOTE**

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Guidance on information requirements and chemical safety assessment

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<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

DOCUMENT HISTORY

Version	Changes	Date
Version 1	First edition	April 2012
Version 2	- Update of section 2.1.1 on sample preparation, to provide specific indications on the parameters for characterisation, pre-requisites and preparation as monitoring awaited for any nanomaterial. - Update of section 2.2.1 on water solubility, to include alternative guidelines that could be used for this endpoint and to flag the non-applicability for insolubility as a waiver for other endpoints - Update of section 2.2.2 on Partition coefficient n-octanol/water, to strengthen the message that guidelines recommended for this endpoint for non-nanomaterials are not applicable for nanomaterials and recommend other parameters that could be considered instead. - Update of section 2.2.4 on Adsorption/desorption, to clarify that the methods recommended in the parent guidance for this endpoint are not applicable for nanomaterials and recommend other parameters that could be considered instead.	Xxxx 2017

PREFACE

The three appendices concerning information requirements (appendices to R7a, R7b and R7c) have been developed in order to provide advice to registrants for use when preparing registration dossiers that cover “nanoforms”.

The advice provided in this document, focuses on specific recommendations for testing materials that are nanomaterials¹. Part of the advice provided is not strictly nano-specific and may for instance be also applicable to other particulate materials (e.g. relevance of dissolution rate). However, when included, we have considered that the issue is especially relevant for nanomaterials and should be part of the nano-specific guidance.

In the absence of any specific recommendation, either because the endpoint is not relevant for nanomaterials, or the guidance already provided is considered to be equally applicable to 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.7a (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: Recommendations 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 See [Recommendation on the definition of nanomaterial](#) adopted by the European Commission

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2 RECOMMENDATIONS FOR PHYSICO-CHEMICAL PROPERTIES

2.1 General remarks

2.1.1 Sample preparation

The following section focuses on preparation of the sample, thus it is implied that choice of the testing material(s) has already been made and that they represent the registered substance and/or the relevant nanoforms.

Sample preparation is widely recognised as one of the most critical steps towards successful characterisation and subsequent testing of nanomaterials. There are many variables to consider when designing a method for sample preparation. Common issues to be considered regarding sample preparation include storage, colloidal and chemical stability of the tested nanomaterial, the chemical composition of the test media, characterisation of stock dispersions and characterisation of samples (prepared from stock dispersions) prior to administration/testing [2].

In order to show that the test material(s) chosen are appropriate to represent the substance and/or the nano(forms) being assessed, some information should be reported at the endpoint study record under the test material information field in IUCLID. The parameters required for the identification of nanoforms should be reported (see [3] for further advice on the type of information required):

- Chemical composition (as described in ECHA Guidance for identification and naming of substances under REACH and CLP)
- Size (as a minimum the D50, but particle size distribution is recommended)
- Shape and aspect ratio
- Surface chemistry

Moreover, the appendix R6-1: Recommendations for nanomaterials applicable to the guidance on QSARs and Grouping of Chemicals [1] provides an approach on how to justify the use of hazard data between nanoforms (and the non-nanoform) of the same substance. The Guidance details some (additional) parameters that may be required to be able to assess whether the available hazard data are applicable for different nanoforms of a substance. The registrant may want to consider characterising the test material taking into account such parameters, in order to be able to follow the above-mentioned guidance. For example, the dissolution rate, surface chemistry and dispersability have been reported as a founding base for the grouping of the nanomaterials [1].

Besides all these parameters, ISO 14887:2007 [4] outlines procedures for the preparation of good dispersions from various powder/liquid combinations for particle size analysis of substances in general. Suggested dispersion procedures for a range of nanomaterials are also emerging in the scientific literature e.g. in [5] and [6]

However, such procedures should be carefully examined to determine if they are adequate for the test material under consideration and modifications may be required for different materials. For example with regard to inhalation toxicity testing, standards are available that outline procedures for the generation of metal nanoparticles using the evaporation/condensation method (ISO 10801:2010 [6]) and support the characterisation of nanoparticles in inhalation exposure chambers [6]).

An important component of sample preparation is the "reliable" sampling. In the reliable sampling the test aliquot used for measurement represents the physical and chemical

characteristics of the entire sample. The characterisation of particle properties like size, form and specific surface area requires very careful sampling and sample splitting practices to be followed. ISO 14488:2007 [4] specifies methods for obtaining a test aliquot from a defined sample of particulate material (powder, paste, suspension or dust) that can be considered to be representative with a defined confidence level and is of particular relevance to the measurement of particle size, size distribution and surface area.

In order to eliminate potential errors in the interpretation of results due to particle contaminants/impurities, data from the characterisation of the test material including its purity and, if technically feasible, quantities of identified contaminants and impurities should be considered prior to the start of a study, consistent with the substance identification requirement.

Also in relation to sample preparation, it is necessary to be aware that aggregates and agglomerates of nanomaterials can form in the dispersion, powder and aerosol forms, and their presence is influenced by a number of factors including the method of synthesis, storage, handling and environmental conditions. Agglomerate means a collection of weakly bound particles. Aggregate means a particle comprising of strongly bound or fused particles (EC Recommendation on the definition of nanomaterial).

The state of agglomeration or aggregation is recognised as an important parameter influencing the interpretation of characterisation and testing of nanomaterials ("as received", "as used", "as dosed / as exposed") and should therefore be considered during sample preparation. A number of measurands have been proposed for assessing agglomeration or aggregation state, including the effective cross-section, determined by measuring aerodynamic/light scattering properties or by electron microscopy ([7], [8]).

Draft test guidelines and a guidance document on agglomeration behaviour and dissolution rate of nanomaterials in aquatic media are under development within OECD and would allow characterisation and quantification of the agglomeration behaviour (see section 2.2.2).

In addition to aggregation and agglomeration, the behaviour of particles in solution presents some additional important aspects and challenges to recognise. In particular, it can be difficult to distinguish between when a nanomaterial is *dispersed* and when it is *dissolved* due to its small particle size. It is important to recognise that solubility and dispersibility are two distinct phenomena. Solubility is the degree to which a material (the solute) can be dissolved in another material (the solvent) such that a single, homogeneous, stable phase results, and is relevant to solids, liquids and gases. Dispersibility is the degree to which a particulate material can be uniformly distributed in another material (the dispersing medium or continuous phase). Historically, the term "dissolved" meant the component of a liquid sample that had passed through a 0.45µm (or similar) filter. However, as (colloidal) dispersions of nanoparticles might also pass through such filters, it is recommended that use of the term "dissolved" should be restricted to the formation of true solutions, and where both liquid and particulates are present the term "dispersed" should be used ([2], [9]).

By applying combination of ultracentrifugation and ultrafiltration techniques it is however possible to measure the amount of truly soluble fraction as can be found in [5] and [10].

A dispersion is a suspension of heterogeneous mixture of nanomaterials comprising a liquid and a finely dispersed solid material, which may falsely have the visible appearance of a solution. Dispersion stability is an important parameter to assess in the context of sample preparation. The dispersion of particles is determined by intermolecular forces involving particle-particle interactions as well as those between the particles and their surrounding matrix. Due to attractive forces (e.g. Van der Waals interactions) particles tend to agglomerate unless stabilised by surface charge or steric effects. As a result, the state of dispersion is dynamic and changes with time to potential dissolution and/or higher agglomeration. Dispersion is determined by interactions between the properties of the nanoparticles and properties of the surrounding matrix. In solution, slight modifications in pH, ionic strength, and concentrations of molecular constituents can significantly alter the dispersion of particles. For aerosolised powders, the situation can be even more complex as the concentration and

diffusion characteristics of the aerosol can cause the state of dispersion to change over time. The state of dispersion is typically assessed using comparative particle size measurements and requires a reliable method of measuring the baseline particle size distribution of the material. By comparing changes in particle size distribution, a qualitative assessment or proxy measure of the state of dispersion can be made. As an example of measurement methods applicable for spherical particles: Zeta potential measurement, combined with Dynamic Light Scattering (DLS), also enables the stability of nanoparticle dispersions to be monitored and a qualitative understanding of the agglomeration process to be achieved. Other methods such as particle tracking analysis can also be used when applicable for the tested substance [11].

2.1.1.1 General considerations for (Eco)-Toxicological testing

In order to start with relevant sample preparation the Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials OECD No. 36 ENV/JM/MONO(2012)40 should be considered. Further guidance on sample preparation may be found from Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines OECD No. 40 ENV/JM/MONO(2014)1 [9], ENV/JM/MONO(2014)1/ADD1 [12], and [13] reflecting the outcome of the discussion of the OECD's work on nanosafety during the Testing Programme of Manufactured Nanomaterials [14].

For example, the following aspects are considered important in sample preparation:

- Characterization of the physicochemical properties of nanomaterials (e.g. particle size distribution, shape, specific surface area, composition, impurities, and surface chemistry) and the state present in the test medium (degree of agglomeration/sedimentation).
- Nanomaterials test item preparation and dispersion (including stability) should take into account the characteristics of the test media [5]. Due to their particular nature in the (eco)toxicological test media, the physico-chemical properties of the nanomaterials as well as the potential (eco)toxicological effects are highly influenced by the interactions with the bio-physicochemical surroundings in these media. Thus, testing should be carried out with accompanying analytics to monitor the exposure concentration. For nanomaterials the use of only chemical analysis is not sufficient, as further explained on dose metrics.
- Sample preparation needs also to be controlled, consistent, relevant, reliable and robust, as the testing stages may include e.g. the use of powder and/or dispersion depending on the end-point, and the test item may have undergone a multi-stage process of preparation.
- Selected sample preparation procedure (and controls, if applied) should be justified and sufficiently reported in the robust study summary.
- Since the most appropriate dose metrics may not be known, the use of other dose metrics than mass-based, such as surface area and particle counts, are to be provided in addition to the mass metrics, when available. These measurements will increase the ability to interconvert doses from mass to particle counts and/ or to surface area and are considered as essential while diminishing the uncertainty related to the conversion when the metrics are used independently and subsequently reducing the amount of testing required.

If a nanomaterial is soluble and has a high dissolution rate (see section 2.2.1) in relevant biological or environmental media, then it is likely to be presented to the test system in its molecular or ionic form and can therefore be expected to elicit the same response as non-nanoscale solubilised substances e.g. the salts of metallic substances used as positive reference versus the metal ionic form stemming from the nanomaterial. If, however, the nanomaterial under investigation is insoluble or sparingly soluble in biological or environmental media, then it will likely be presented to the test system in a particle form. In which case, the advice provided in *Appendices on Recommendations for nanomaterials applicable to Chapters R.7a (this document), R.7b and R.7c* will apply.

1 In addition, nanoparticles may interact with the liquid phase components, partially or totally
2 yielding soluble or dispersed transformation products (as well as some solubilised nanomaterial
3 itself) that may influence the overall toxicity and fate processes. This possibility needs to be
4 taken into account when selecting the media and procedures as well as in the assessment of
5 the result of any test ([2], [15]).

6 Other important considerations to take into account during sample preparation include the
7 influence of contaminants (including biological contaminants) and impurities on
8 (eco)toxicological test results. For example, metallic impurities such as Co and Ni catalysts
9 used in the production process of the nanoparticles were shown to inhibit hatching in zebrafish
10 embryos (e.g. [16]).

11 Of particular concern also for nanomaterials, is the influence of endotoxin on certain testing
12 results. Endotoxin (lipopolysaccharide) is a constituent of the outer cell wall of Gramnegative
13 bacteria and as such is found ubiquitously within the environment. Endotoxin however can
14 generate a range of toxic effects either at the whole organism level causing responses such as
15 fever, 'endotoxin shock' and death, or at the cellular level via the triggering of inflammatory
16 cascades leading to the secretion of pro-inflammatory mediators.

17 Due to the potent response endotoxin can generate in biological assays, toxicity testing of a
18 contaminated test sample may lead to a confounding of results (including a potential false
19 positive). As such the establishment of the presence or level of endotoxin in a test sample is
20 an important preliminary undertaking during the preparation of a sample for toxicological
21 testing. Endotoxin can be measured using in vitro methods such as the macrophage activation
22 test, which has been validated by European Committee on Validation of Alternative Test
23 Methods and proposed as reliable method for determining the pyrogenicity of engineered,
24 research-grade nanomaterials [17]. International standards are available for the testing of
25 nanomaterials [18]. Although issues regarding contamination are not nano-specific, the
26 increased relative surface area of nanophase systems compared to other particles or aqueous
27 substances means that the possible amounts of adsorbed endotoxin (e.g. grams adsorbed
28 endotoxin per gram of material) are significant [19].

29
30 The existence of false negatives has also to be accounted for, for instance in cases where
31 exposure of the organism is underestimated (e.g. Ames test, insoluble particles etc). Due to
32 differences in fate and behaviour, testing of nanomaterials and traditional chemicals in
33 different test environments, a testing strategy/decision tree approach on dispersion,
34 dissolution, dispersion stability and aggregation recommended in OECD No. 40 [9] may be
35 considered. This approach takes into account e.g. the effects of pH, DOM, NOM/proteins, and
36 ionic strength and should be accounted for until specific test guidelines and guidance
37 documents developed by OECD are made publicly available (see 2.1.1). Considerations and
38 measurement of dissolution rate and dispersion stability in the media will not only help to find
39 the appropriate testing strategy and test conditions, but also help in the interpretation of the
40 results. This information would also be useful for nanomaterial grouping and read-across [1].
41

2.2 Specific advice for endpoints

2.2.1 Water solubility

Water solubility is covered in Section R.7.1.7 of the parent guidance. In the case of nanomaterials it is necessary to take into account that water solubility has the potential to increase for materials in the nano-size range due to their decreasing particle size and it may also be affected by their shape and surface coating. For nanomaterials, the dissolution rate and dispersion also play an important role in mobility of the substance. However, it can be difficult to distinguish between when a substance is dispersed and when it is dissolved due to its small particle size. It is important to recognise that solubility and dispersibility are different and distinct phenomena, with different implications on testing and characterisation, and it is important to differentiate between them. This situation is not unique to nanomaterials, and indeed the parent guidance already highlights that “measurement of the solubility of sparingly soluble compounds requires extreme care to generate saturated solutions of the material without the introduction of dispersed material”. However, this problem may be further amplified in the case of sparingly soluble nanomaterials. Further information on these issues is provided in section 2.1.1 on Sample Preparation. It should also be ensured that no undissolved material contributes to what is being measured as being dissolved material.

The OECD has examined the applicability of its test guidelines for nanomaterials and OECD publications have stated that OECD TG 105 [20] (Water solubility) is not always appropriate for testing of nanomaterials [12].

This is the case when the substance in question has low water solubility, and where the possibility of generating dispersion also exists. Measurement of water solubility using OECD TG 105 guideline may still be of value for nanomaterials that are water soluble and have a high and fast dissolution rate.

2.2.1.1 Other guidelines and protocols for solubility

Measurement of the rate and extent of dissolution, as supporting information and/or as an alternative method when OECD TG 105 is not applicable, is highly recommended as dissolution rate in relevant biological and environmental media is relevant knowing that this affects the bioavailability of substances in the (biological) environment (OECD No. 62 [21]). For instance, the data on dissolution rate may be useful to determine what type of testing is required for aquatic toxicity testing (see for instance section 1.2.1 in *Appendix R.7-1 to Chapter R.7b*). OECD 29 allows to test dissolution and transformation for test duration varying between 1 up to 28 days with a usual duration of 7 days being applied. When choosing the testing material for this endpoint, please take into account that testing the smallest particle size (as recommended by the guideline) may not be adequate.

OECD 62 instead, is mainly focusing on dissolution rate and setting qualitative thresholds of high or > 70 % of dissolution into another form, moderate between 10 and 70 %, low >1 and below 10 % and negligible < 1%, all estimated for a test duration of 7 days.

The OECD 29 on transformation/dissolution testing protocol [22] provides advice to determine the rate and extent to which metals and sparingly soluble metal compounds can produce soluble available ionic and other metal bearing species in aqueous media. The measurement of rate and qualitative thresholds developed further in OECD No. 62 provides with further advice and on how to apply the transformation/dissolution protocol on metallic nanomaterials for the moment. Furthermore, there are two additional test guidelines for determining the dissolution rate of nanomaterials under developments within OECD that will be applicable instead of the OECD 29 once they are available:

- Test Guideline for the Dissolution Rate of Nanomaterials in the Aquatic Environment ,
- Guidance Document on Agglomeration and Dissolution behaviour of Nanomaterials in

Aquatic Media.

Measurements of agglomeration and aggregation can also be useful together with the dissolution rate using the guidance documents above once available.

2.2.1.2 (In)solubility as a waiver

In the parent Guidance Section R.7.1.7.1 it is noted that water insolubility is used as a regulatory trigger for waiving certain physicochemical and ecotoxicological endpoints. However for nanomaterials insolubility alone is not relevant as a justification for test waiving. The high insolubility of a nanomaterial does not necessarily indicate that toxicity is unlikely. Exposure cannot be excluded, as even an insoluble nanomaterial may be bioavailable to the test organisms due to nano-specific properties e.g. size and dispersibility. Furthermore, Test Guidelines not appropriate for highly insoluble substances may be applicable for nanomaterials with specific adaptation.

Taking into account the nano-specific properties and constraints in assessing the solubility of nanomaterials by currently available standard methods such as OECD TG 105 (Water solubility), waiving the information requirement based on high insolubility should always be accompanied with robust technical and scientific justification.

For instance, further information on dissolution, agglomeration and sedimentation could be used as a part of the weight of evidence to justify an alternative testing strategy (e.g. including a sediment toxicity test).

2.2.2 Partition coefficient n-octanol/water

Section R.7.1.8.3. of the parent guidance, includes information regarding experimental data on n-octanol/water partition coefficient including testing methods. The n-octanol/water partition coefficient (K_{ow}) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of the largely immiscible solvents n-octanol and water. In a two-phase system, nanoparticles behave differently from organic molecules. The fate of nanoparticles may not be predicted by equilibrium partitioning ([23], [24]) as nanoparticles cannot reach thermodynamic equilibrium by distributing between two phases, water and n-octanol, due to their particulate nature. Therefore, OECD TGs recommended in the parent ECHA Guidance for partition coefficient n-octanol/water, i.e. OECD TG 107, OECD TG 117 and OECD TG 123, are in most cases not applicable to nanoparticles ([7], [9], [13]). Results might be impacted upon by the presence of a colloidal suspension, which could be present if the manufactured nanomaterial does not completely dissolve ([2], [9]).

Nevertheless, if it is shown that the nanomaterial is fast and highly dissolved, and presence of particles can be excluded the parent guidance will apply. Taking into account the above, 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 (see section 2.2.1).

The use of n-octanol/water partition coefficient (K_{ow}) might lead to erroneous interpretation of the environmental fate or bioconcentration [23]. Taking into account the nano-specific properties and constraints in assessing the n-octanol/water partition coefficient (K_{ow}) of the nanomaterials by currently available standard methods, waiving the information requirement based on n-octanol/water partition coefficient should always be accompanied by a robust technical and scientific justification on the applicability of the used test method (e.g. nanomaterial being water soluble or have a high and fast dissolution rate).

In the parent Guidance section 7.1.8.3, "Difficult to test substances", it should be noted that due to the small particle size of nanomaterials, it can be difficult to distinguish between when a substance is dispersed and when it is dissolved. It is important to recognise that solubility and dispersibility are two distinct phenomena and it is important to differentiate between them. Further information on these issues is provided in section 2.1.1. on Sample Preparation.

2.2.2.1 Other guidelines and protocols for K_{ow}

Regarding nanomaterials, currently there are no proper standard methods for fate descriptors to predict the behaviour and transport of nanomaterials in the environment and biological media as alternative to n-octanol/water partition coefficient ([23], [24]). There are, however, alternative means to equilibrium partitioning that may be used to predict fate and transport of the nanomaterials in the environment and organisms. Agglomeration, aggregation, deposition and attachment are considered to be informative means to predict behaviour of the nanoparticles ([23], [25], [26], [27]). Alternative fate descriptors for nanoparticles are further discussed in section 2.2.4 on adsorption/desorption.

In the OECD, there is ongoing activity on development of the following test guidelines for determining the agglomeration behaviour of nanomaterials:

- Test Guideline for Agglomeration Behaviour of Nanomaterials in different Aquatic Media [28]
- Guidance Document for Agglomeration behaviour and Dissolution rate of Nanomaterials in Aquatic Media.

Assessment of agglomeration of nanomaterials is to be conducted in accordance with OECD TG when available².

Other non-testing methods can also be considered in case the K_{ow} measurement is not applicable. The list and details on the models and specific parameters under development are available in Appendix 1.

² The draft is available at: <http://www.oecd.org/env/ehs/testing/test-guidelines-for-comments-section3-degradation-and-accumulation.htm>

2.2.4 Adsorption/desorption

In the parent guidance, the methods for determining this endpoint are shown in Table R.7.1-14 Methods for the measurement of adsorption. Adsorption/desorption measurements are used in fate modelling to indicate which compartment in the environment will be exposed the most or might need to be considered in hazard and risk assessment. This measurement helps to determine in which environmental compartment (e.g. soil, sediment or water) the substance is most likely to end up and whether is likely to be mobile or immobile in the environment. For instance, high adsorption to soil would show that both soil and sediment are the most relevant environmental compartments to be considered in hazard assessment.

Adsorption³ is temporary (reversible) or permanent bonding between the substance and a surface. With regard to nanomaterials, the distribution coefficient K_d may have to be based on actual testing since estimations of K_d derived from the organic carbon-water partition coefficient (K_{oc}) and the octanol-water partition coefficient (K_{ow}) might not be applicable when it comes to nanomaterials. K_d measurement is also based on the assumption of thermodynamic equilibrium between liquid and solid phase. Equilibrium partitioning does not apply to undissolved nanoparticles ([13], [23], [24];) as described in section 2.2.2 Partitioning coefficient n-octanol/water. Hence, nanoparticles do not always form solutions, but colloidal dispersions, which are multiphase systems and thermodynamically unstable. Thus, nanoparticle dispersions can be kinetically stable for a long period of time (typically through electrostatic or steric stabilization) but they will never reach thermodynamic equilibrium and consequently cannot be equilibrated with an additional phase [24], [29].

Therefore, nanoparticles strive to reduce their surface energy by attaching to each other. This attachment can be:

- homoagglomeration/aggregation between the particles of the same nanomaterial, or,
- heteroagglomeration/aggregation with other particles or with e.g. organic matter, or
- to the interface between phases (deposition or attachment).

Because of our inability to accurately quantify the physicochemical forces contributing to particle attachment, this step is typically described by an empirical parameter termed the particle attachment efficiency (α) that needs to be determined in agglomeration (hetero-agglomeration) or deposition experiments [23], [29].

OECD TG 106 Adsorption – Desorption Using Batch Equilibrium Method is not applicable to nanomaterials because it is currently not possible to differentiate between adsorbed or aggregated/agglomerated nanoparticles settled during the centrifugation step, and a new TG needs to be developed ([9], [30]). However, if it is shown that a nanomaterial is fast and highly dissolved, it can be assessed as traditional chemicals and the parent guidance will apply.

It is necessary to take into account the nanoparticle specific properties and constraints in assessing the adsorption/desorption of nanoparticles by currently available methods based on K_d derived from the organic carbon-water partition coefficient (K_{oc}) and the octanol-water partition coefficient (K_{ow}) such as OECD TG 106. As a result, waiving the information requirement based on low adsorption/desorption should always be accompanied with robust technical and scientific justification on the applicability of the used test method.

2.2.4.1 Other guidelines and protocols for K_{oc} or K_d

OECD TG 312 Leaching in Soil Columns [31] studies the mobility and leaching of the test substance into deeper soil layers or ground water. Using OECD TG 312, K_d values can be derived from column leaching studies and these are considered generally applicable for nanomaterials.

Alternative approaches and measurements to describe adsorption/desorption of nanoparticles

³Please note that distribution/partitioning does not equal adsorption, and neither does sorption, which consists of adsorption and absorption phenomena.

1 based on the determination of retention of nanomaterials in soils by screening techniques [24]
2 or K_d and other equations based on colloidal suspensions or particles not reaching
3 thermodynamic equilibrium have been discussed.

4 Other parameters than K_d or $\log K_{oc}$ could be considered for nanoparticles such as
5 (hetero)agglomeration, aggregation, particle attachment and removal. Agglomeration
6 behaviour has been identified as an important parameter affecting the environmental
7 behaviour of nanomaterials. The agglomeration parameter depends on the physicochemical
8 characteristics of the nanomaterial itself, the physicochemical characteristics of the suspension
9 medium, suspension preparation, concentration of the nanomaterial and concentration of other
10 substances and particles in the suspension. The agglomeration behaviour is controlled by
11 kinetics (energy barriers) rather than thermodynamic equilibrium. Therefore information on
12 the agglomeration and aggregation behaviour of nanomaterials is recommended to be
13 generated before their further testing.

- 14 • The Draft OECD TG on Agglomeration Behaviour of Nanomaterials in Different aquatic
15 Media [28] is available at [http://www.oecd.org/env/ehs/testing/test-guidelines-for-](http://www.oecd.org/env/ehs/testing/test-guidelines-for-comments-section3-degradation-and-accumulation.htm)
16 [comments-section3-degradation-and-accumulation.htm](http://www.oecd.org/env/ehs/testing/test-guidelines-for-comments-section3-degradation-and-accumulation.htm).

17 Determination of sorption is critical to assessing amounts of nanomaterials released to surface
18 waters, and to soils and sediments ([32], [33], [34]; [35]). Particle attachment and removal
19 from wastewater can be used as another alternative approach to predict sorption of
20 nanomaterials.

- 21 • For example OECD TG 303A “Aerobic Sewage Treatment Simulation Test” may be used
22 as an indirect measurement to predict sorption of nanomaterials into sludge by
23 determining the distribution of the nanomaterials between sludge and effluent.

24 These alternative approaches are still under development and further validation is needed.
25 Whenever they will be available, they will be recommended for providing suitable alternative
26 information on the sorption and agglomeration/aggregation of nanomaterials. The pre-
27 assessment of dissolution rate and agglomeration behaviour of nanomaterials is needed in
28 order to proceed with any alternative measurement of their attachment or deposition ([9],
29 [13], [36]).

30 Other non-testing methods can also be considered in case the K_{oc} and K_d measurement are not
31 valid. A list of available models to predict alternative fate descriptors for nanomaterials are
32 available in Appendix 1.
33
34
35

Appendix 1. Models for fate for nanomaterials

There is on-going research in development of modelling tools to assess the fate of nanomaterials. The list of methods provide by Table 1 is not exhaustive and shows methods based on attachment affinity and dissolution rate of nanomaterials. Further information on methods that may be used to predict fate and transport of nanomaterials in the environment and organisms can be found at for instance at [37].

Further information on the models and status of validation can be found in the referenced publications for each model.

Table 1: Overview of some models for fate for nanomaterials

Model	Overview	Output	Link to the model tools	References
SimpleBox4nano (SB4N): Classical multimedia mass balance modeling system	The model expresses engineered nanoparticles (ENP) transport and concentrations in the environmental compartments (air, water, soil, etc.) accounting processes such as aggregation, attachment, and dissolution. The model solves simultaneous mass balance equations.	The output is mass concentrations of ENPs as free dispersive species, heteroaggregates with natural colloids, and larger natural particles in each compartment in time and at steady state.	http://www.rivm.nl/simplebox	[38]
NanoDUFLOW: Spatiotemporally explicit hydrological model	Feedbacks between local flow conditions and engineered nanoparticles (ENPs) fate processes, such as homo- and heteroaggregation, resuspension and sedimentation, are modelled.	The outputs are the concentrations of all ENP forms and aggregates in water and sediment in space and time, and retention.	DUFLOW Modelling Studio (v3.8.7) software package with a set of specific processes defined by the user via the NanoDUFLOW submodel.	[39]
Steady-state distribution model	Multimedia model was developed using nanospecific process descriptions such as homo- and heteroaggregation, dissolution and sedimentation to estimate the steady-state distribution	The output is nanoparticle / mass concentrations in water and sediment, and its distance from the source.	As a first case study in Praetorius et al., [27] a river model was used.	[27]

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