

Guidance on information requirements and chemical safety
assessment

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

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May 2016



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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>

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1 **DOCUMENT HISTORY**

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Version	Changes	Date
Version 1	First edition	April 2012
Version 2	<ul style="list-style-type: none"> • 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

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1 **PREFACE**

2 The three appendices concerning information requirements (appendices to R7a, R7b and R7c)
3 have been developed in order to provide advice to registrants for use when preparing
4 registration dossiers for nanomaterials¹.

5 In the absence of any specific recommendation, either because the endpoint is not relevant for
6 nanomaterials (e.g. flash point or surface tension), or the guidance already provided is
7 considered to be equally applicable to nanomaterials or because more research is needed
8 before developing advice, no additional guidance for the endpoint has been included in this
9 appendix.

10 This appendix intends to provide advice specific to nanomaterials and does not preclude the
11 applicability of the general principles given in Chapter R.7a (i.e. the parent guidance). The
12 parent Guidance applies when no specific information for nanomaterials has been given in this
13 appendix.
14

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

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 preparation. Common issues to be considered regarding sample preparation include storage and 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 [1]. Guidance on sample preparation considering the physico-chemical characterisation of nanomaterials, covering properties such as particle size distribution, shape, specific surface area, octanol-water partition coefficients, degree of agglomeration and dispersion behaviour, is available [1] ISO 14887:2000 [2] 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. 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 [3]) and support the characterisation of nanoparticles in inhalation exposure chambers [3]).

An important component of sample preparation is the need to have “reliable” sampling, such that 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 [2] 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. An agglomerate is defined as a collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components. An aggregate is a particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components (ISO 27687:2008 [4]).

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 ([5]. [6]) OECD [5] [6] suggests for nanomaterials with

1 a non-zero width of the distribution that the degree of agglomeration should be characterised.
2 Other measurands include the average agglomeration number (AAN), which is derived from
3 the ratio of the volume based median particle size to the average equivalent spherical volume
4 derived from BET gas adsorption.

5 In addition to aggregation and agglomeration, the behaviour of particles in solution presents
6 some additional important aspects and challenges to recognise. In particular, it can be difficult
7 to distinguish between when a nanomaterial is *dispersed* and when it is *dissolved* due to its
8 small particle size. It is important to recognise that solubility and dispersibility are two distinct
9 phenomena. Solubility is the degree to which a material (the solute) can be dissolved in
10 another material (the solvent) such that a single, homogeneous, stable phase (a suspension
11 down to the molecular level) results, and is relevant to solids, liquids and gases. Dispersibility
12 is the degree to which a particulate material can be uniformly distributed in another material
13 (the dispersing medium or continuous phase). Historically, the term “dissolved” meant the
14 component of a liquid sample that had passed through a 0.45µm (or similar) filter. However,
15 as (colloidal) dispersions of nanoparticles might also pass through such filters, it is
16 recommended that use of the term “dissolved” should be restricted to the formation of true
17 solutions, and where both liquid and particulates are present the term “dispersed” should be
18 used ([1], [7]).

19 A dispersion is a suspension of discrete insoluble particles in a fluid, which may falsely have
20 the visible appearance of a solution (i.e. the product of the conversion of a solid substance to
21 liquid form by mixture with a solvent). Dispersion stability is an important parameter to assess
22 in the context of sample preparation. The dispersion of particles is determined by
23 intermolecular forces involving particle-particle interactions as well as those between the
24 particles and their environment. Due to attractive forces (e.g. Van der Waals interactions)
25 particles tend to agglomerate unless stabilised by surface charge or steric effects. As a result,
26 the state of dispersion is dynamic and determined primarily by the environment of the
27 nanoparticles. In solution, slight modifications in pH, ionic strength, and concentrations of
28 molecular constituents can significantly alter the dispersion of particles. For aerosolised
29 powders, the situation can be even more complex as the concentration and diffusion
30 characteristics of the aerosol can cause the state of dispersion to change over time.

31 The state of dispersion is typically assessed using comparative particle size measurements and
32 requires a reliable method of measuring the baseline particle size distribution of the material.
33 By comparing changes in particle size distribution, a qualitative assessment or proxy measure
34 of the state of dispersion can be made. Zeta potential measurement, combined with Dynamic
35 Light Scattering (DLS) also enables the stability of nanoparticle dispersions to be monitored
36 and a qualitative understanding of the agglomeration process to be achieved.

37 2.1.1. General considerations for (Eco)-Toxicological testing

38 In order to start with relevant sample preparation the Guidance on Sample Preparation and
39 Dosimetry for the Safety Testing of Manufactured Nanomaterials OECD No. 36
40 ENV/JM/MONO(2012)40 should be considered. Further guidance on sample preparation may
41 be found from Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test
42 Guidelines OECD No. 40 ENV/JM/MONO(2014)1 [7], ENV/JM/MONO(2014)1/ADD1 [8], and
43 Rasmussen et al. (2016) reflecting the outcome of the discussion of the OECD’s work on
44 nanosafety during the Testing and Assessment of Manufactured Nanomaterials – programme.
45

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

- 47
- 48 • Characterization of the physical-chemical properties of nanomaterials.
- 49 • Test item preparation and dispersion (including dispersion stability) should take into
50 account the characteristics of the test media [9](Hartmann et al. 2015). Due to their
51 particular nature in the (eco)toxicological test media, the physico-chemical properties of
52 the nanomaterials as well as the potential (eco)toxicological effects are highly
53 influenced by the interactions with the bio-physico-chemical surroundings in these
54 media. Thus, testing should be carried out with accompanying analytics to monitor the
55 exposure concentration.

- 1 • Sample preparation needs to be controlled, consistent, relevant, reliable and robust as
2 the testing stages may include e.g. the use of powder and/or dispersion depending on
3 the end-point, and the test item may have undergone a multi-stage process of
4 preparation.
- 5 • Since the most appropriate dose metrics may not be known, the use of other dose
6 metrics than mass-based, such as surface area and particle counts, are to be provided
7 in addition to the mass metrics, when available. These measurements will increase the
8 ability to interconvert doses from mass to particle counts and/ or to surface area and
9 are considered as essential.

10
11 If a nanomaterial is soluble, with a high dissolution rate in biological or environmental media,
12 then it is likely to be presented to the test system in its molecular or ionic form and can
13 therefore be expected to elicit the same response as bulk (non-nanoscale) solubilised
14 substances. If, however, the nanomaterial under investigation is insoluble or sparingly soluble
15 in biological or environmental media, then it will likely be presented to the test system in a
16 particle form.

17 In addition, nanoparticles may interact with the liquid phase components, partially or totally
18 yielding soluble or dispersed transformation products (as well as some solubilised nanomaterial
19 itself) that may influence the overall toxicity and fate processes. This possibility needs to be
20 taken into account when selecting the media and procedures as well as in the assessment of
21 the result of any test ([1], [10]).

22 Other important considerations to take into account during sample preparation include the
23 influence of contaminants (including biological contaminants) and impurities on
24 (eco)toxicological test results. For example metallic impurities such as Co and Ni catalysts
25 used in the production process of the nanoparticles have shown to be the cause of adverse
26 effects on a test species (e.g. [11]).

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

33 Due to the potent response endotoxin can generate in biological assays, toxicity testing of a
34 contaminated test sample may lead to a confounding of results (including a potential false
35 positive). As such the establishment of the presence or level of endotoxin in a test sample is
36 useful as a preliminary undertaking during the preparation of a sample for toxicological
37 testing. International standards are available for the testing of nanomaterials (ISO
38 29701:2010 [12]) although issues regarding endotoxin contamination are not necessarily
39 nano-specific and are equally relevant for other particles or aqueous substances undergoing
40 toxicological evaluation.

41
42 Due to differences in fate and behaviour testing of nanomaterial and traditional chemicals in
43 different test environments, a testing strategy/decision tree approach on dispersion,
44 dissolution, dispersion stability and aggregation recommended in OECD No. 40 [7] may be
45 considered. This approach takes into account e.g. the effects of pH, DOM, NOM/proteins, and
46 ionic strength and should be accounted for until specific test guidelines and guidance
47 documents developed by OECD are made publicly available (see 2.1.1). Such considerations
48 and measurements of dispersion, dissolution and stability in the media will provide additional
49 information on the behaviour of the nanomaterials and help to find the appropriate testing
50 strategy and test conditions.
51

1 2.2 Specific advice for endpoints

2 2.2.1 Water solubility

3
4 Water solubility is covered in Section R.7.1.7 of the parent guidance. In the case of
5 nanomaterials it is necessary to take into account that water solubility has the potential to
6 increase for materials in the nano-size range due to their decreasing size and it may also be
7 affected by their shape and surface coating. For nanomaterials, the dissolution rate and
8 dispersion also play an important role in mobility of the substance. However, it can be difficult
9 to distinguish between when a substance is dispersed and when it is dissolved due to its small
10 particle size. It is important to recognise that solubility and dispersibility are different and
11 distinct phenomena, with different implications on testing and characterisation, and it is
12 important to differentiate between them. This situation is not unique to nanomaterials, and
13 indeed the parent guidance already highlights that “measurement of the solubility of sparingly
14 soluble compounds requires extreme care to generate saturated solutions of the material
15 without the introduction of dispersed material”. However, this problem may be further
16 amplified in the case of sparingly soluble nanomaterials. Further information on these issues is
17 provided in section 2.1.1 on Sample Preparation. It should also be ensured that no undissolved
18 material contributes to what is being measured.

19
20 The OECD has examined the applicability of its test guidelines for nanomaterials. A
21 preliminary review of the applicability of OECD test guidelines concluded that OECD guideline
22 105 might be applicable under some circumstances or to some classes of nanomaterials
23 (ENV/JM/MONO(2009)21 [6]) . However, later publications have considered that OECD TG 105
24 [13] (Water solubility) is not appropriate for testing of nanomaterials [8]. This is the case
25 when the substance in question has low water solubility, and where the possibility of
26 generating dispersion also exists. Measurement of water solubility using this guideline may still
27 be of value for nanomaterials that are water soluble or have a high dissolution rate.

28
29 Measurement of rate and extent of dissolution is highly recommended (OECD No. 62 [14]).
30 Dissolution/dissolution rate in relevant biological and environmental media is relevant as this
31 affects the bioavailability of substances in the (biological) environment. The dissolution rate
32 depends on the chemical composition, particle size, coating, stability, manufacturing process,
33 and biological environment. The relevance of the different media depends on the actual route
34 of exposure and/or the environmental compartment under evaluation [15].

35
36 In the OECD, there is ongoing activity on development of the following test guidelines for
37 determining the dissolution rate of nanomaterials:

- 38
- Test Guideline for the Dissolution Rate of Nanomaterials in the Aquatic Environment
 - Guidance Document for Agglomeration behaviour and Dissolution rate of Nanomaterials in Aquatic Media.
- 39
40

41 Where tests on the substance are required to generate information on intrinsic properties of
42 the substance, they must be conducted in accordance with the test methods laid down in a
43 Commission Regulation or in accordance with other international test methods recognised by
44 the Commission or the Agency as being appropriate. Assessment of dissolution rates of
45 nanomaterials must be conducted in accordance with OECD TG when available.

46 In the parent Guidance Section R.7.1.7.1 it is noted that water insolubility is used as a
47 regulatory trigger for waiving certain physicochemical and ecotoxicological endpoints. However
48 for nanomaterials insolubility alone is not always relevant as a justification for test waiving.
49 The high insolubility as a mitigating factor indicating that e.g. toxicity is unlikely, might not be
50 true for nanomaterials due to nano-specific properties and exposure ([16], [15]. Furthermore,
51 Test Guidelines not appropriate for highly insoluble substances may be applicable for
52 nanomaterials with specific adaptation.

53
54 Taking into account the nano specific properties and constraints in assessing the solubility of

1 nanomaterials by currently available standard methods such as OECD TG 105 (Water
2 solubility), waiving the information requirement based on high insolubility should always be
3 accompanied with robust technical and scientific justification on the applicability of the test
4 method used.

6 **2.2.2 Partition coefficient n-octanol/water**

7 Section R.7.1.8.3 includes information regarding experimental data on n-octanol/water
8 partition coefficient including testing methods. The n-octanol/water partition coefficient (K_{ow}) is
9 defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase
10 system consisting of the largely immiscible solvents n-octanol and water. In a two-phase
11 system, nanoparticles behave differently from organic molecules. The fate of nanoparticles
12 may not be predicted by equilibrium partitioning ([17]; [18]) as nanoparticles cannot reach
13 thermodynamic equilibrium by distributing between two phases, water and n-octanol, due to
14 their particulate nature. Therefore, OECD TGs recommended in the parent ECHA Guidance for
15 partition coefficient n-octanol/water, OECD TG 107, OECD TG 117 and OECD TG 123 are not
16 applicable to nanoparticles ([19]; [7], [5]). Results might be impacted upon by the presence
17 of a colloidal suspension, which could be present if the manufactured nanomaterial does not
18 completely dissolve ([1], [7]). Measurement of n-octanol/water partition coefficient may still
19 be of value for nanomaterials that are water soluble or have a high dissolution rate.

20 The use of n-octanol/water partition coefficient (K_{ow}) might lead to erroneous interpretation of
21 the environmental fate or bioconcentration [17]. Taking into account the nano-specific
22 properties and constraints in assessing the n-octanol/water partition coefficient (K_{ow}) of the
23 nanomaterials by currently available standard methods, waiving the information requirement
24 based on n-octanol/water partition coefficient should always be accompanied by a robust
25 technical and scientific justification on the applicability of the used test method.

26 In the parent Guidance section 7.1.8.3, "Difficult to test substances", it should be noted that
27 due to the small particle size of nanomaterials, it can be difficult to distinguish between when a
28 substance is dispersed and when it is dissolved. It is important to recognise that solubility and
29 dispersibility are two distinct phenomena and it is important to differentiate between them.
30 Further information on these issues is provided in [Section 2.1.2](#) on Sample Preparation.

31 Regarding nanomaterial, currently there are no proper standard methods for fate descriptors
32 to predict the behaviour and transport of nanomaterials in the environment and biological
33 media as alternative to n-octanol/water partition coefficient ([17]; [18]). There are, however,
34 alternative means to equilibrium partitioning that may be used to predict fate and transport of
35 the nanomaterials in the environment and organisms. Aggregation, deposition and attachment
36 are considered to be informative means to predict behaviour of the nanoparticles ([17], [20],
37 [21]). Alternative fate descriptors for nanoparticles are further discussed in section 2.2.4 on
38 adsorption/desorption.

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

- 41 • Test Guideline for Agglomeration Behaviour of Nanomaterials in different Aquatic Media
- 42 • Guidance Document for Agglomeration behavior and Dissolution rate of Nanomaterials
43 in Aquatic Media.

44
45 Assessment of agglomeration of nanomaterials must be conducted in accordance with OECD
46 TG when available.
47

2.2.4 Adsorption/desorption

Adsorption is caused by temporary (reversible) or permanent bonding between the substance and a surface. In the parent guidance, the methods for determining this endpoint are shown in Table R.7.1-14 Methods for the measurement of adsorption. With regard to nanomaterials, the distribution coefficient K_d has 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}) are not applicable when it comes to nanomaterials. Equilibrium partitioning does not apply to nanoparticles ([17]; [18]; [19]) as described in section 2.2.2 Partitioning coefficient n-octanol/water. Hence, nanoparticles do not 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 can consequently not be equilibrated with an additional phase [18].

Therefore, nanoparticles cannot reach thermodynamic equilibrium by distributing between phases but they can reduce their surface energy by attaching to each other (aggregation) 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 aggregation or deposition experiments [17].

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 nanoparticles and settled nanoparticles, and a new TG needs to be developed ([7]; [22]).

It is necessary to take into account the nano 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.

OECD TG 312 Leaching in Soil Columns [23] 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 based on the determination of retention of nanomaterials in soils by screening techniques [18] or K_d and other equations based on colloidal suspensions or particles not reaching thermodynamic equilibrium have been discussed.

Other parameters than K_d or $\log K_{oc}$ could be considered for nanoparticles such as agglomeration, particle attachment and removal. Agglomeration behaviour has been identified as an important parameter affecting the environmental behaviour of nanomaterials. The agglomeration parameter depends on the physicochemical characteristics of the nanomaterial itself, the physicochemical characteristics of the suspension medium, suspension preparation, concentration of the nanomaterial and concentration of other substances and particles in the suspension. The agglomeration behaviour is controlled by kinetics (energy barriers) rather than thermodynamic equilibrium. Therefore information on nanomaterials' agglomeration behaviour is recommended to be generated before their further testing. The Draft OECD TG Agglomeration Behaviour of Nanomaterials in Different aquatic Media is available.

Determination of sorption is critical to assessing amounts of nanomaterials released to surface waters, and to soils and sediments ([24], [25], [26]; [27]). Particle attachment and removal from wastewater can be used as another alternative approach to predicts sorption of nanomaterials. For example OECD TG 303A "Aerobic Sewage Treatment Simulation Test" may

1 be used as an indirect measurement to predict sorption of nanomaterials into sludge by
2 determining the distribution of the nanomaterials between sludge and effluent.

3 All these approaches are still under development and further validation is needed. Whenever
4 they will be available, they will be recommended for providing suitable alternative information
5 on the sorption and agglomeration/aggregation of nanomaterials. The pre-assessment of
6 dissolution rate and agglomeration behaviour of nanomaterials is needed in order to proceed
7 with any alternative measurement of their attachment or deposition ([7], [28], [19]).

8

9

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