

GUIDANCE

Guidance on information requirements and chemical safety
assessment

Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7b Endpoint specific guidance

Draft (Public) Version 2.0

January 2017



1 **LEGAL NOTE**

2

3 This document aims to assist users in complying with their obligations under the REACH
4 Regulation. However, users are reminded that the text of the REACH Regulation is the only
5 authentic legal reference and that the information in this document does not constitute legal
6 advice. Usage of the information remains under the sole responsibility of the user. The
7 European Chemicals Agency does not accept any liability with regard to the use that may be
8 made of the information contained in this document.

9

Guidance on information requirements and chemical safety assessment

Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7b - Endpoint specific guidance

Reference: ECHA-XXXXXX-EN

ISBN: XXXXXX

Publ.date: XXXXXX

Language: EN

© European Chemicals Agency, 2017

Cover page © European Chemicals Agency

Reproduction is authorised provided the source is fully acknowledged in the form
"Source: European Chemicals Agency, <http://echa.europa.eu/>", and provided written
notification is given to the ECHA Communication Unit (publications@echa.europa.eu).

If you have questions or comments in relation to this document please send them (indicating
the document reference, issue date, chapter and/or page of the document to which your
comment refers) using the Guidance feedback form. The feedback form can be accessed via
the ECHA Guidance website or directly via the following link:

[https://comments.echa.europa.eu/comments cms/FeedbackGuidance.aspx](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

1 **DOCUMENT HISTORY**

2

Version	Changes	Date
Version 1	First edition	April 2012
Version 2	<ul style="list-style-type: none"> • New advisory note (section 1.1) on testing for ecotoxicity and fate, to provide overall advice for conducting ecotoxicity and environmental fate testing for nanomaterials • Update of section 1.2.1 on aquatic pelagic toxicity, to clarify that high insolubility cannot be used as a waiver and to include further recommendations on the text to be performed for this endpoint • Update of section 1.2.2. on Toxicity for sediments organisms to provide advice on spiking methods and include applicability of available OECD guidelines • Update of section 1.2.3 on degradation/ biodegradation to clarify that waivers for hydrolysis and degradation simulation testing are not applicable as sole evidence, provide advice on photocatalytic degradation and general advice on performing the tests <p>Please note that the numbering of the sections has changed, the section numbers above refer to the updated numbering of the guidance</p>	Xxxx 2017

3

4

5

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 that cover “nanoforms”.

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

10 In the absence of any specific recommendation, either because the endpoint is not relevant for
11 nanomaterials, or the guidance already provided is considered to be equally applicable to
12 nanomaterials or because more research is needed before developing advice, no additional
13 guidance for the endpoint has been included in this appendix.

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

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

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

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

27

28

¹ See [Recommendation on the definition of nanomaterial](#) adopted by the European Commission

Table of contents

1		
2		
3	DOCUMENT HISTORY	3
4	PREFACE	4
5	1 RECOMMENDATIONS FOR ECOTOXICOLOGICAL ENDPOINTS for NANOMATERIALS:	6
6	1.1 General Advice on how to perform nanomaterials ecotoxicity and fate testing	6
7	1.1.1 Non-testing data.....	7
8	1.2 Specific advice for endpoints.....	8
9	1.2.1 Aquatic pelagic toxicity	8
10	1.2.1.1 Test guidelines specifics for aquatic toxicity.....	10
11	1.2.2 Toxicity for sediment organisms	11
12	1.2.2.1 Test guidelines for sediment toxicity	13
13	1.2.3 Degradation/Biodegradation/Transformation	13
14	1.2.3.1 Biodegradation	13
15	1.2.3.2 Abiotic degradation	14
16	1.2.3.3 Transformation	14
17	1.2.3.4 Surface chemistry in degradation/transformation testing	15
18	1.2.3.5 Test guidelines for degradation/biodegradation.....	16
19		
20		

1 RECOMMENDATIONS FOR ECOTOXICOLOGICAL ENDPOINTS for NANOMATERIALS:

1.1 General Advice on how to perform nanomaterials ecotoxicity and fate testing

This section provides general advice for ecotoxicological and fate testing regardless of the test compartment or endpoint. Endpoint specific guidance is provided under corresponding endpoint specific sections.

This section summarises the advice (sampling, preparation for testing, testing itself and reporting the results) provided in the documents listed below and in the publications by Petersen et al. [2] and Rasmussen et al. [3].

- OECD No.36 : Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials [4];
- OECD No.40: Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. Expert Meeting Report [5];
- OECD No.40 (1): Addendum to Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. Expert Meeting Report [6];
- OECD No.62: Considerations for Using Dissolution as a Function of Surface Chemistry to Evaluate Environmental Behaviour of Nanomaterials in Risk Assessments. A Preliminary Case Study Using Silver Nanoparticles [7];
- OECD No.64: Approaches on Nano Grouping/ Equivalence/ Read-Across Concepts Based on Physical-Chemical Properties (Gera-Pc) for Regulatory Regimes [8].

The guidance detailed below should be taken into account when results on nanoform(s) is reported (when relevant for the endpoint) in the technical dossier.

Prerequisites

It is advised to consider the following issues when the nanomaterials are tested:

- Define representative controls for the test (e.g. for metal oxide nanomaterials, metal salt solutions as benchmarks)
- Dissolution rate and potential ion release (see section 2.2.2.1 in *Appendix R.7-1 to Chapter R.7a of the IR&CSA Guidance for dissolution criteria: high, moderate, low or negligible*).
- Agglomeration behaviour, degradation and transformation (using the OECD TG on agglomeration behaviour in aquatic media and the corresponding GD under development, OECD No. 40 [5])
- Justification of the selected exposure regimes (e.g. test duration, static or flow through, exposure route, etc.).

The exposure media and conditions of the test should be consistent and repeatable (as explained in the section on sample preparation of *Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a - Endpoint specific guidance* [9]).

- Define the frequency of the measurements of concentration of the test material to detect any decrease in concentration or transformation during the test.

- 1 • Quantify the concentration changes due to e.g. aggregation and sedimentation or
2 transformation with relevant metrics to provide reliable exposure concentrations during
3 the testing
4
- 5 • When performing a test, besides the use of mass metric, other nano-specific measurands
6 (e.g. specific surface area, volume) have to be considered giving the measurement
7 techniques are applicable (see for instance [10], [11]).
8
9

10 Preparations before testing

11 The following considerations need to be taken into account when preparing the test:

- 12 • Stock dispersion²:
 - 13 ○ Dispersion preparation used for the stock dispersion should be reported
 - 14 ○ Direct application of the stock dispersion vs. preparation of a stock suspension
15 should be reported.
 - 16 ○ The level of purity³ needed for the test material stock dispersion should be
17 considered.
 - 18 ○ Dispersion stability in stock dispersion ([2], [11])
- 19 • Test media and possible interactions with the test material:
 - 20 ○ Selection of the dispersion protocol appropriate for the test media and the test
21 material (as mentioned above). The dispersion method should not change the
22 characteristics of the test material (See for instance [11]).
23
 - 24 ○ The agglomeration behaviour and dissolution of the nanomaterial in the specific test
25 media used and its potential effects on exposure (See OECD No. 36 [4] and OECD
26 No. 40 [5] and addendum [6]), where relevant. Apply the test guidelines and
27 guidance (once available) for the Agglomeration Behaviour and Dissolution Rate of
28 Nanomaterials in the Aquatic Media (See also [12], [13]).
29
 - 30 ○ Consider particle stability in the test medium. This means performing the test as
31 required by the guideline but without the test organism(s) to clarify the interactions
32 between the test material and the test media. Potential interactions (See for
33 instance [10]) of the test material with the test media may be:
 - 34
 - 35 ▪ complexation with the nutrients;
 - 36 ▪ interaction with dissolved or natural organic matter (DOM/NOM);
 - 37 ▪ Surface affinity;
 - 38 ▪ Precipitation or sedimentation of the test material.
39

40 The OECD Guidance on Aquatic (and Sediment) Toxicology Testing of Nanomaterials will provide
41 further advice on these issues once available.

42 1.1.1 Non-testing data 43

² Dry spiking method is discussed in section 2.1.1.

³ In this context purity may refer to chemical purity and also to biological contamination

1 Although the use of non-testing approaches such as (Q)SAR approaches in addressing data
2 gaps for nanomaterials is still limited, non-testing methods are recommended if and when they
3 provide relevant and reliable information and are applicable as they significantly reduce the
4 amount of testing required. However, the use of non-testing approaches for nanomaterials in
5 deriving an assessment of hazard for environment must be thoroughly and scientifically
6 justified. Further non testing approaches are explained in Appendix R.6-1: Recommendations
7 for nanomaterials applicable to the Guidance on QSARs and Grouping which provides an
8 approach on how to justify the use of hazard data between nanoforms (and the non-nanoform)
9 of the same substance. When considering the read-across and/or grouping between nanoforms
10 of different substances the advice provided in the ECHA Guidance Chapter R.6 on QSARs and
11 Grouping of the Chemicals [10] together with the advice provided in its nanospecific appendix
12 [1] could be considered.

13 **1.2 Specific advice for endpoints**

14 The parent R7b Endpoint specific guidance section R7.8 includes sections for aquatic pelagic
15 toxicity, toxicity to sediment organisms and activated sludge. The approaches and methods
16 described for these endpoints in the parent guidance are in principle applicable also for
17 nanomaterials.

18 Nevertheless, the recommendations set out in *Appendix R.7-1 to Chapter R.7a* [9], section
19 2.1.1 need to be taken into consideration, especially with regard to dispersion preparations,
20 method of nanomaterials introduction, storage and stability of test material, chemical
21 composition of the relevant test media, characterisation of stock dispersions, characterization
22 of samples (prepared from stock dispersions prior to administration/testing and if possible
23 during and/or at the end of the test) and different measurement protocols.

24 If it is proven that the nanomaterials under investigation are fast and highly dissolved, they
25 would be assessed as traditional chemicals (See section 2.2.1 in *Appendix R7-1 Chapter R7a*).
26 In that case, for ecotoxicological and fate endpoints, the advice provided in the parent
27 guidance will apply. The only nanospecific tests would be the physico-chemical ones including
28 data on dissolution rate in the specific test media.

29 **1.2.1 Aquatic pelagic toxicity**

30 When performing aquatic toxicity testing for nanomaterials, the advice provided in this section
31 should be followed instead of that in Section 7.8.1 of the parent guidance. It is recommended
32 that the following points are taken into account:

- 33 • Sample preparation (section 2.1.1 in *Appendix R7-1 Chapter R7a*)
- 34 • General advice on how to perform nanomaterial ecotoxicity and fate testing (see
35 section 1.1)
- 36 • Applicability of the test guidelines
- 37 • Specific considerations for waiving based on high insolubility, as per REACH column 2
- 38 • Preference for long-term testing
- 39 • Endpoint-specific recommendations

40 In addition to the general advice given above, the following specific advice for aqueous
41 experiments should be followed, implemented and reported:

- 42 • Use of synthetic dispersants is not recommended to prepare the stock dispersion or
43 solution for aquatic toxicity testing, unless they are constituents of the registered
44 substance (product formulation), in which case the bioassay should be conducted with
45 the as-produced material [2]
- 46 • Provide the media characteristics (e.g. pH, ionic strength, natural organic matter

1 (NOM), humic acid).

- 2 • Testing to be carried out with accompanying analytics to monitor the exposure
3 concentration (for example: sedimentation rate [2], [10], [14]).

4 The OECD TGs and their recognised equivalents for algae, aquatic invertebrates and fish are
5 considered generally applicable for nanomaterials [3]. However, contrary to the parent
6 guidance R7b Section 7.8.2., this adaptation is generally not acceptable for nanomaterials
7 because the adaptation to waive aquatic toxicity tests based on substance being highly
8 insoluble in water cannot be used without proper and scientifically robust justification (as
9 highlighted in Appendix R.7-1 to Chapter R.7a, section 2.2.1.). As explained above, low
10 solubility does not automatically result in limited exposure of nanomaterials in the aquatic
11 environment. Furthermore, in most cases, the dissolution rate should be considered instead of
12 solubility for nanomaterials. Based on the results of the dissolution rate test, the following
13 options are possible:

- 14 • The nanomaterial is dissolved and has a high dissolution rate in relevant media (in
15 OECD No.62 [7]). However, "fast dissolution" should be assessed with respect to the
16 test duration, e.g. a material can be considered as dissolving fast for a long-term fish
17 test but not for the activated sludge inhibition study. In case high solubility and fast
18 dissolution can be demonstrated, there are no further considerations specific to
19 nanomaterials to be taken into account, and the parent guidance can be followed.
- 20 • The nanomaterial does not dissolve fast e.g. conforms to moderate or lower dissolution
21 rate criteria. Thus, the registrant is advised to preferably perform long-term toxicity
22 testing instead of testing for short-term toxicity⁴ depending on the type of testing
23 and experimental set-up applied (in particular for *Daphnia* and Fish long term testing is
24 advised).⁵ For these testing considerations the ITS from the parent guidance (section
25 R.7.8.2) can be followed.
- 26 • If acute toxicity testing is chosen, the conditions and test settings must be assessed in
27 order to prove that the exposure concentration is adequate and duration is long
28 enough to capture potential toxic effects. If further testing is needed the ITS from the
29 parent guidance should be followed (section R 7.8.2).
- 30 • Long-term toxicity testing (including Algae) otherwise, must be considered for
31 nanomaterials, as already specified if they conform to the properties outlined in the
32 parent guidance Section R.7.8.2., i.e. poor water solubility and for the nanomaterials
33 to the negligible, low or moderate dissolution rate criteria (See Appendix R.7-1 to
34 Chapter R.7a, section 2.1.1) [9].
- 35
- 36 • In case the nanomaterial behavioural properties (e.g. dissolution rate negligible,
37 aggregation or agglomeration) lead to reduced aquatic and relevant sediment
38 exposure, then testing strategy favouring sediment toxicity test can be considered.
- 39 • In any cases where the long term toxicity tests would be chosen as aquatic toxicity
40 tests and to be performed, a testing proposal must be submitted by the registrant for
41 both invertebrates and vertebrates testing as per REACH information requirements
42 Article 40 on Annex IX section 9.1.5 and 9.1.6.
- 43

⁴ If the dissolution rate is slow, short term testing will not provide reliable results due to limited exposure. For nanomaterials that do not dissolve 'quickly' a chronic test is more appropriate to capture effects after dissolution than an acute test. Kinetics of uptake and biodistribution are the key factors in this respect, not only for the dissolved material, but also for the nanoparticles themselves.

⁵ In cases where nanoparticles dissolve over time in media, acute toxicity tests may be conducted not only using a freshly prepared suspension in test medium, but also an aged suspension where NPs are added to the media 1-3 days prior to testing, depending on the shelf life of the media [10]. This aging step may increase or decrease toxicity, which regardless provides important weight of evidence on toxicity. Furthermore, it allows for processes of aggregation and dissolution of nanoparticles in aqueous suspensions to stabilize prior to exposure. However, testing solely with aged particles does not fulfil the information requirement for short aquatic toxicity.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

1.2.1.1 Test guidelines specifics for aquatic toxicity

When aquatic toxicity tests are performed for nanomaterials, some additional parameters and testing specifications could be considered (and further reported, if applied), as specified (per endpoint) below:

- For Fish testing (OECD TG 210 [15]):
 - mechanical effects, e.g. blocking of respiratory organs, decrease of ventilation rate, gill pathologies and blocking of digestive tract, [16], [17],
 - activity levels of relevant antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione-S-transferase (GST), [18], [19], [20]
 - fish mucus secretion [16],
 - fish brain pathology [17],
 - animal behaviour [15],
 - histopathology of fish [17],
 - the potential effects of photoactivity or catalytic properties of the nanomaterial on toxicity [19], [20], when relevant (for instance a depigmentation or other stress indicators)
- For Daphnids testing (OECD TG 202 [21] and OECD TG 211 [22]):
 - the role of nutrient depletion effect (for long-term evaluation) should be considered in relation to the test setup to avoid potential artefacts in the interpretation of the results
 - sex-ratio for Daphnia (number of males and females as per OECD TG 211 [22])
 - any behavioural observations [21], [22], [23]
 - mechanical effects of the nanomaterial (e.g. adherence to the organism, blocking of oxygen diffusion or digestive tract, [14], [24]), and
 - the potential effects of photoactivity or catalytic properties of the nanomaterial on toxicity [25], [26], [27] when relevant
- For Algae testing (OECD TG 201 [28]):
 - quantification of effects on colour or shading, using protocols such as the ones developed by [24] and [29].
 - mechanical effects of the nanomaterial (e.g. adherence to the organism)
 - the type of agitation used in the test setup (stirring/shaking) for preventing/slowing down sedimentation
 - fluorescence measurement of chlorophyll extracts (considered as the most reliable way of measuring algal biomass for testing effects of nanomaterials on algae growth (OECD No. 40 [5], OECD No. 40(1) [6], [30]) or pigments quantification [29].

- 1 • autofluorescence of the tested nanomaterial to avoid misinterpretation of chlorophyll
2 extracts based on adsorption/interaction with nanomaterials [30], and for instance
3 testing under different light regimes, additional endpoints to improve reliability of
4 the results
- 5 ○ Example; in addition to the algal growth rate inhibition or carbon-
6 assimilation another endpoint for more subtle effects to the individual algal
7 cells, such as membrane damage and oxidative stress.⁶
- 8 • when relevant, the potential effects of photoactivity or catalytic properties of the
9 nanomaterial on toxicity,

10 For activated sludge inhibition:

- 11 • In the parent Guidance R7b Section R.7.8.17, Information requirements for toxicity to
12 STP microorganisms, it is stated that STP toxicity testing is not needed if there are
13 mitigating factors such as a high insolubility that would limit the exposure. This
14 adaptation is generally, not acceptable for the activated sludge toxicity testing of
15 nanomaterials or, as explained above, for the aquatic toxicity testing of nanomaterials
16 in general.

17

18 **1.2.2 Toxicity for sediment organisms**

19 Situations in which the equilibrium partitioning method (EPM) can be applied in estimating
20 toxicity to sediment organisms are presented in parent guidance Sections R. 7.8.9.1 and
21 R.7.8.10.1, covering use of non-testing data on toxicity to sediment organisms. Regarding
22 nanomaterials, estimates based on results from “equilibrium partitioning methods” (i.e. based
23 on thermodynamic equilibrium) are limited to the distribution of a substance in molecular
24 form. In the case of nanoparticles, the partitioning method is not recommended, as it may
25 underestimate exposure in soil and sediment environments and overestimate the exposure in
26 water.

27 There are no estimation methods available for particle distribution in sediment, so this has to
28 be dealt with on a case-by-case basis. With regard to nanomaterials, the recommendations set
29 out in the OECD Guidance Manual for testing [31] and updated Guidance Notes on Sample
30 Preparation and Dosimetry for nanomaterials [4] need to be taken into consideration, including
31 the further advice from Appendix R.7-1 to Chapter R.7a, section 2.1.1 and the ones above
32 mentioned in this chapter section 1.1 and 1.2. Especially recommendations in regard to
33 methods of suspension, method of nanomaterials introduction, storage and stability of test
34 material, chemical composition of the test media, characterisation of stock dispersions, as well
35 as characterization of samples (prepared from stock dispersions) prior to
36 administration/testing and possibly during and at least at the end of the test are important to
37 be addressed. Many of the considerations for aquatic toxicity testing for nanomaterials, as
38 detailed above in section 1.2.1.1, are also relevant to sediment tests [2].

39 Nanomaterial suspensions are often not stable in natural waters (e.g. due to agglomeration
40 and sedimentation) and will have along residence time [32]. Therefore, there is often relevant
41 exposure to sediment compartment. Hazard assessment in the sediment compartment can in
42 many cases provide more relevant information than the pelagic aquatic hazard assessment (

⁶ A possibility for nanomaterials with fast acting toxic mechanisms or substantial dissolution in media is to perform a short-term 2h ¹⁴C-assimilation test, potentially combined with an aging step. Carbon assimilation is likely less influence by shading than growth rate. Also, less interference with the scintillation counting is expected, compared to the spectrophotometric determination of algal pigments often used in growth rate inhibition tests. Ultimately, the use of single endpoint testing is sensitive to artefacts and misinterpretations, especially where the testing prerequisite of solubility and stability is violated, and there is little knowledge on the toxic mode of action. (Sørensen 2016)

1 [2], [3]). In case the nanomaterial behavioural properties and uses lead to reduced aquatic
2 and relevant sediment exposure, as described above in this document and under R7 a section
3 2.2.1.2., then an alternative testing strategy including sediment toxicity test can be
4 considered.

5 Some added complications are that nanomaterial interactions with sediments can significantly
6 alter their properties, alike metals and metal oxides for which these aspects have been
7 discussed in *Appendix R7.13-2 to the Guidance on IR&CSA* [33]. Additionally, the methods for
8 quantifying nanomaterial characteristics in sediments (e.g. concentration) are very limited.
9 Current sediment toxicity standard methods acknowledge significant uncertainty regarding test
10 substance homogeneity, exposure, bioavailability and synergisms. Nevertheless, the
11 consistency of sediment toxicity bioassays can still be generally improved by implementing
12 standards for preparation and experimental set-up as indicated above (section 1.1 and 1.2).
13 For instance, the use of a standardized (e.g., OECD) freshwater sediment in nanomaterial
14 spiking studies would reduce variability in bioassay results relative to the use of field-collected
15 sediments because sediment-specific factors (e.g., organic carbon concentration) that can
16 influence toxicity assay results are controlled.

17 Two types of spiking methods for nanomaterials have been applied in sediment toxicity
18 testing:

19 (1) direct addition to the sediment of dry nanomaterials (dry spiking) or dispersed
20 nanomaterials (wet spiking) nanomaterials to the sediment, followed by homogenization and

21 (2) indirect addition of nanomaterials to the overlying water, followed by subsequent settling
22 of the nanomaterials to the surface sediment.

23

24 The test material will be better dispersed in sediment if the spiking is done with an already
25 dispersed solution rather than with dry material⁷. This is related to general difficulties
26 regarding homogenizing chemicals into sediments. If a nanomaterial is added to sediment in
27 powder form (undispersed), it is likely that substantial clumping of particles within the
28 sediment occurs, resulting in greater heterogeneity and therefore greater variability among
29 bioassay test replicates. When the test substance is mixed with the sediment (direct sediment
30 spiking) it is recommended to use dispersed nanomaterial preparation instead of dry stock test
31 material.

32 Indirect spiking of overlying water has also challenges. Indirect spiking is followed by settling
33 of the nanomaterials to the sediment and will result in non-homogeneous distribution of the
34 nanoparticles in the sediment (gradient from surface to deeper layers) and therefore increases
35 the heterogeneity of the subsamples. This should be acknowledged when indirect spiking is
36 applied and variability of the exposure in each subsample should be minimised. The optimal
37 spiking method depends on both the test material and the test method. It will depend on the
38 physicochemical properties of the nanomaterial, the target concentration, the medium, and the
39 bioassay method selected, and preliminary data gathered prior to the test.

40 Further to the spiking method, equilibration time between performing the test and sediment
41 spiking depends on the type of nanomaterial and knowledge on its behaviour parameters such
42 as agglomeration, aggregation and sedimentation. For example, if one uses an equilibration
43 time of 48 hours, the test could be considered a worst-case scenario with the highest

⁷ According OECD Guidance 40, it is recommended to use the same aqueous solution for the sediment and the aquatic toxicity testing.

1 bioavailability, as no pseudo-equilibrium stage will be reached in such a short time [2], unless
2 it is proven otherwise.

3 Technical challenges in nanomaterials characterization methods may limit the detection of
4 nanoparticles and the determination of particle characteristics in sediment. Certain
5 measurements may still be performed, such as using ICP-MS to determine the total elemental
6 concentration of metal and metal-oxide nanomaterials. As an example, the use of ICP-MS may
7 be combined with separation techniques (e.g. field-flow-fractionation) enabling single particle
8 measurements and more detailed information on the metal/metal-oxide nanoparticles. It is
9 practical to take samples for such measurements from the whole sediment, sediment
10 porewater, and overlying water at least at test initiation and termination, as recommended in
11 current OECD sediment testing guidance. However, nanomaterial-specific modifications of
12 porewater separation methods may be needed in order to yield accurate results [2]. Such
13 methods could be applied to measuring nanomaterials in the different compartments of the
14 test and would allow a better distinction of the source/type of toxicity, depending on where the
15 nanomaterial distributes.

16 **1.2.2.1 Test guidelines for sediment toxicity**

17

18 The following OECD TGs are reviewed and considered generally applicable for nanomaterials:
19 OECD TG 225 (Sediment Lumbriculus Assay [34]) and OECD TG 218 [35] and 219 [36]
20 (Sediment-Water Chironomid Toxicity Using Spiked Sediment and Sediment-Water Chironomid
21 Toxicity Using Spiked Water respectively). In addition OECD TG 233 Sediment-Water
22 Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment could also be
23 applied for nanomaterials.

24 Whatever the test method and the method of spiking chosen, the equilibration time before
25 performing the testing, the sampling method and the analysis technique and frequency have to
26 be reported.

27 Furthermore, the reporting of information on the preparation sampling and experimental setup
28 need to be provided as explained in *Appendix R.7-1 to Chapter R.7a* [9], section 2.1.1. In
29 addition the parameters specified in this appendix from sections 1.1 and 1.2 on aquatic pelagic
30 testing need also to be followed (such as pH, ionic strength, natural organic matter (NOM) and
31 humic acid). All this information would also need to be reported together with the methods
32 analysis and test results as explained above and in Section 1.1.

33

34 **1.2.3 Degradation/Biodegradation/Transformation**

35 Degradation is a process that can result in the loss or transformation of a substance in the
36 environment. Environmental compartments to be considered in risk assessment are water,
37 sediment, and soil. In addition, degradation and transformation of a substance in sewage
38 treatment plant plays a key role in fate and exposure assessment. If the
39 degradation/transformation rate is fast this should be taken into account in hazard, exposure
40 and risk assessment.

41

42 **1.2.3.1 Biodegradation**

43 The degradation process can be abiotic or biotic. Biodegradation is a biological process in
44 which organic substances are decomposed by microorganisms. A baseline for biodegradation in
45 the context of the available biodegradation test guidelines is that the test material is based on
46 organic carbon chemistry (for bulk chemicals as well as for nanomaterials). This leads to the
47 conclusion that the concept of biodegradability as applied to organic substances has limited or

1 no meaning for inorganic substances, including inorganic nanomaterials e.g. Ag, TiO₂, CeO₂,
2 nZVI, ZnO, CuO and QDs [37]. In addition, many of the carbon-based nanomaterials such as
3 carbon nanotubes (CNTs) and carbon black are considered to show inorganic characteristics.
4 There is however evidence on biotic degradation of carbon-based nanomaterials, single-walled
5 carbon nanotubes (SWCNT), multiwalled carbon nanotubes (MWCNTs) and fullerenes (C₆₀) by
6 oxidative enzymes ([38], [39], [40]). On the other hand, for MWCNTs there are results
7 indicating no degradation by oxidative enzymes alone but up to 7 % mineralisation by a mixed
8 bacterial culture at 39° C resulting several degradation products [41]. Even if the extent of
9 biodegradation of carbon-based nanomaterials in natural environmental conditions is
10 considered limited, the above-described studies indicate that potential for biological
11 degradation in relevant environmental conditions remains to be established [37]. Thus for
12 carbon-based nanomaterials it is recommended that performing a degradation study is always
13 considered. If a carbon-based nanomaterial is considered not degradable without testing, this
14 needs to be justified.

15 Ready biodegradation testing is most likely not relevant for inorganic nanomaterials which do
16 not contain carbon, at least in terms of ultimate biodegradation parameters (O₂ consumption,
17 CO₂ production, and DOC removal). Regarding carbon-based nanomaterials of inorganic
18 nature, even though their degradation potential may be limited, it is at least theoretically
19 possible that ultimate biodegradation based on O₂ consumption or CO₂ production could be
20 detected in ready biodegradation tests. In addition, there can be issues with the applicability of
21 the test methods to nanomaterials, e.g. due to the stringent test conditions. Therefore, for
22 carbon-based nanomaterials of inorganic nature, ready biodegradability testing may be less
23 relevant compared to organic substances. However, despite these limitations, even when the
24 pass level for ready biodegradability is not met, ready biodegradation test or other screening
25 level biodegradation test might give valuable information on extent of degradation.
26 Furthermore the potential for release of degradation/transformation products is recommended
27 to be taken into account in any degradation assessment of nanomaterials, including those of
28 inorganic nature.

29 **1.2.3.2 Abiotic degradation**

30 In the parent guidance R7b section 7.9.3.1, abiotic processes such as hydrolysis, oxidation and
31 photolysis are considered important transformation routes for chemicals in water, soil and
32 sediment. Hydrolysis might be relevant to consider also for some nanomaterials and/or
33 coatings. The oxidation-reduction process does play a key role in the behaviour of some
34 nanomaterials such as Ag, CuO and ZnO. Measurement of redox potential is important for
35 nanomaterials that can participate in electron transfer and uptake. This phenomenon is
36 important also in relation to interaction with environmental media ([42], [43]). Photochemical
37 transformation is relevant for some nanomaterials as it may lead to changes in the
38 nanomaterial's surface properties, or degradation of the coating or degradation of the
39 nanoparticle itself ([37], OECD No. 63 [44] and OECD No. 65 [45]). These changes may lead
40 to altered behaviour and hazard and are therefore important to be considered in
41 degradation/transformation assessment. It is recommended to consider also alternative means
42 of which some are described below to clarifying the environmental fate of the nanomaterial.

43

44 **1.2.3.3 Transformation**

45 Transformation of the nanomaterial may be chemical, biologically mediated or interaction with
46 macromolecules in the test media or in the environment. Nanomaterials having high surface to
47 volume ration makes the transformation of high relevance for their fate. The available
48 methods to study the transformation of nanomaterials in relevant environments is still scarce,
49 standard protocols are not available and many methods are still under development. Therefore
50 clear recommendations on the test methods in many cases may not be at this point given.

1 However, in the absence of standardised and/or quantitative methods, qualitative assessment
2 may provide valuable information on the fate of nanomaterials. Transformation processes
3 considered relevant for nanomaterials are described below (not exclusive).

4 Reduction and oxidation are the main chemical transformation processes. Nanomaterials may
5 undergo oxidation and reduction in all environmental compartments. Light-catalysed redox
6 reactions may also be important transformation processes affecting e.g. oxidation stage and
7 generation of reactive oxygen species (ROS). Dissolution and sulfidation may also be
8 considered as chemical transformation processes relevant for nanomaterials. In biologically
9 mediated transformation chemical transformations are mediated by living organisms in living
10 tissue (intra end extracellular) and environmental media via redox-labile enzymes,
11 cytochromes, and intracellular ROS production (hydroxyl radicals or H₂O₂). For example it has
12 been demonstrated that biological oxidation can result carboxylation of CNTs or formation of
13 an insoluble metal oxide shell. Interactions with macromolecules e.g. proteins, polysaccharides
14 and NOM, may alter the behaviour of nanomaterials as they may be adsorbed to the surface of
15 the nanoparticle forming a "corona" around the nanoparticle. This corona may then change for
16 instance the size, mobility and surface characteristics of the nanoparticles leading to different
17 behaviour and biological responses compared to particles without the corona. For example the
18 dissolution rate, entry to cells, accumulation and ROS production might be effected [46].

19 The following key transformation processes influencing environmental fate and behaviour have
20 been considered relevant for nanomaterials (in [37], [43], [46] and [47]):

- 21 • Oxidation-reduction
- 22 • Photochemical degradation
- 23 • Biotransformation
- 24 • Speciation – complexation
- 25 • Loss of coating
- 26 • Adsorption/desorption of (other) substances
- 27 • Corona formation

28 The processes listed above take into account processes on the level of an individual particle
29 (e.g. photochemical transformation), interactions between particles (e.g. corona formation),
30 and interactions of particles with solid surfaces and with other substances (e.g.
31 adsorption/desorption). When quantitative analysis of these parameters is not possible due to
32 lack of applicable methods also qualitative assessment may provide valuable information in
33 fate assessment of nanomaterials. The parent guidance highlights the challenges and case
34 specificity in using the information on photochemical degradation in classification and
35 chemicals safety assessment [48].

36 Water solubility and the octanol-water partitioning tests may not be appropriate for
37 nanomaterials, as explained in the *Appendix R.7-1, Chapter R.7a*, sections 2.1.1 and 2.2.2.
38 Therefore, the above-mentioned transformation processes are recommended to be considered
39 in the testing strategy for nanomaterials degradation. This approach is also supported by
40 Rasmussen et al. [3] proposing a fate decision tree logic and testing strategy taking into
41 account the dissolution rate and agglomeration behaviour when testing the nanomaterials.

42 **1.2.3.4 Surface chemistry in degradation/transformation testing**

43 If the nanomaterial is coated or functionalized with organic and potentially biodegradable
44 materials, then biodegradation tests would need to be performed for the coatings alone or for
45 the coated nanomaterials. If the test is performed with the coated nanomaterial, the amount
46 of carbon needs to be high enough to allow reliable detection of the e.g. released carbon
47 dioxide or consumed oxygen. In addition, potential effects of surface modifications on
48 degradation/transformation may need to be considered, as it has been shown that surface
49 modifications may have an effect on the degradation/transformation properties of
50 nanomaterials, e.g. MWCNTs in [49]. In case the coating is degraded/transformed, the

1 observed changes and their potential effects on the behaviour, fate and toxicity need to be
2 considered within the endpoint specific testing regimes. For instance, knowledge on the
3 degradation / transformation on the coating may influence the testing strategy. Depending on
4 whether the coating of the nanomaterial is stable or not, it may be more relevant to perform
5 hazard test on the coated nanomaterial, the non-coated or both. (See for instance *Part D of*
6 *the Guidance on IR&CSA*)

7 8 **1.2.3.5 Test guidelines for degradation/biodegradation**

9 **Abiotic degradation**

10 The chemical structure of the nanomaterial and whether it contains functional groups which
11 could be subject to hydrolysis dictate whether a hydrolysis test is necessary or appropriate. If
12 the nanoparticle is coated or functionalised, then abiotic degradation, e.g. hydrolysis of the
13 substance, must be considered.

14 OECD TG 316 (Phototransformation of Chemicals in Water – Direct Photolysis), though not
15 specifically validated for nanomaterials, may be applied to assessing the photocatalytic
16 degradation or photolysis of nanomaterials ([37], OECD 63 [44] and OECD 65 [45]).

17 **Biodegradation**

18 Concerning information on degradation/biodegradation (Section R.7.9.3 of parent guidance
19 R7b section R7.9), it should be noted that the OECD biodegradability test methods have been
20 developed and validated principally for the assessment of organic compounds. Many
21 nanomaterials are inorganic and even many carbon-based nanomaterials are of inorganic
22 nature, and therefore the biodegradation test methods currently recommended in the parent
23 guidance may be inadequate for predicting their long-term fate of nanomaterials in the
24 environment.

25 The OECD TGs for ready biodegradability and simulation tests in water, soil and sediment
26 listed in the parent guidance are in principle applicable for testing the degradation of an
27 organic nanomaterial, organic coated/functionalised nanomaterial, organic coating or
28 functionalisation agent. If the degradation of an organic coating or functionalisation agent is
29 tested on its own, the potential differences in the degradation/transformation potential
30 compared to when bound to the particle should be taken into account. The guidance provided
31 in OECD No. 36 [4], and OECD No. 40 [5], in this Appendix and in Appendix R7-1 to Chapter
32 R7a on sample preparation, dispersion and dissolution should be followed before proceeding
33 with fate testing.

34 Determination of sorption (see section 2.2.4 Appendix R7-1 to Chapter R7a) is also critical for
35 assessing amounts of nanomaterials released to surface waters, and to soils and sediments ([50], [51], [52], [53]). Some biodegradation test guidelines could be applied for
36 nanomaterials to provide information on distribution of the nanomaterials, acknowledging that,
37 nanomaterials may not sorb to solid phases (e.g. in soil, sediment or sludge) according to the
38 equilibrium kinetics that apply to traditional chemicals [3].

40 The OECD TG 303A “Aerobic Sewage Treatment Simulation Test” has been found to be useful,
41 in particular for assessing the distribution of nanoparticles in sewage treatment plants e.g.
42 [54] with the following proposals for modifications:

- 43 • The dosing of nanoscale suspensions should be made separately from that of the
44 organic synthetic wastewater in order to avoid any agglomeration of the particles.
45 (Unless it is the intention of the study to investigate such processes).
- 46 • The use of synthetic drinking water for preparation of the test suspension instead of tap
47 water to allow better comparability of test results.

- 1 • The test should be performed under nitrifying conditions to also assess the impact of
- 2 nanomaterials on the nitrifying microorganisms, besides the effects on the organic
- 3 carbon degrading microorganisms in the activated sludge.
- 4 • The determination of the filterable solids in the effluents of the laboratory sewage
- 5 treatment plant (LSTP), nature and partitioning of the nanoscale particles in the effluent
- 6 (filtration/centrifugation) is recommended.
- 7 • The calculation of an overall mass balance should be provided with the test results.

8 A new test guideline is under development in OECD that could be used to estimate the particle
9 attachment and removal efficiency from nanomaterials in the wastewater treatment.

10 **Other methods**

11 Alternative protocols can provide information on the abiotic degradation/transformation of
12 nanomaterials when very low or negligible degradation is observed in degradation
13 measurements.

- 14 • Oxidation-reduction
- 15 • Photochemical degradation (e.g. OECD TG 316)
- 16 • Dissolution (see section 2.2.1 in appendix R7-1 to chapter R7a [9])
- 17 • Adsorption - desorption (currently no standard method available, see section 2.2.4 in
- 18 appendix R7-1 to chapter R7a [9])
- 19 • Agglomeration (see section 2.2.1 and 2.2.2 in appendix R7-1 to chapter R7a [9])
- 20 • Aggregation (see section 2.2.1 and 2.2.2 in appendix R7-1 to chapter R7a [9])
- 21 • Biotransformation
- 22 • Speciation – complexation

23 As described above recommendations for applicable test methods for the above parameters
24 are not provided. Applicability of available methods is dependent of the type of nanomaterial,
25 many methods are still under developments and standard methods are not available. However,
26 this type of information, even if qualitative, is recommended to be used as part of the Weight
27 of Evidence on degradation assessment of nanomaterials to strengthen the conclusion on
28 (bio)degradability/transformation and fate ([4], [5], [37]). One of the intention of these
29 alternative methods and data is to feed in more realistic estimations of the levels and nature of
30 environmental (and human) exposure to the nanomaterial, as well as to allow appropriate
31 testing of the form of nanomaterial in which exposure predominantly occurs.

1 **REFERENCES**

2

- [1] ECHA, “[DRAFT] Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance on QSARs and Grouping,” [Online]. Available: <http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach>.
- [2] E. J. Petersen, S. A. Diamond, A. Kennedy, G. Goss, K. Ho, J. Lead, S. Hanna, N. Hartmann, K. Hund-Rinke, B. Mader, N. Manier, P. Pandard, E. Salinas and P. Sayre, “Adapting OECD Aquatic Toxicity Tests for Use with Manufactured Nanomaterials: Key Issues and Consensus Recommendations,” *Environmental Science & Technology*, vol. 49, no. 16, p. 9532–9547, 2015.
- [3] K. Rasmussen, M. Gonzalez, P. Kearns, J. Riego Sintes, F. Rossi and P. Sayre, “Review of achievements of the OECD Working Party on Manufactured Nanomaterials' Testing and Assessment Programme. From exploratory testing to test guidelines,” *Regulatory Toxicology and Pharmacology*, vol. 74, p. 147–160, 2016.
- [4] OECD, “Series on the Safety of Manufactured Nanomaterials- No. 36. Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterial. Series on the Safety of Manufactured Nanomaterials. ENV/JM/MONO(2012)40,” 2012. [Online]. Available: <http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm>.
- [5] OECD, “Series on the Safety of Manufactured Nanomaterials- No. 40. Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. Expert Meeting Report ENV/JM/MONO(2014)1,” 2014. [Online]. Available: <http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm>.
- [6] OECD, “Series on the Safety of Manufactured Nanomaterials- No. 40. Addendum to Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. Expert Meeting Report ENV/JM/MONO(2014)1/ADD,” [Online]. Available: <http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm>.
- [7] OECD, “Series on the Safety of Manufactured Nanomaterials- No. 62 Considerations for Using Dissolution as a Function of Surface Chemistry to Evaluate Environmental Behaviour of Nanomaterials in Risk Assessments. ENV/JM/MONO(2015)44.,” 2015. [Online]. Available: <http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm>.
- [8] OECD, “Series on the Safety of Manufactured Nanomaterials- No.64-Approaches on Nano Grouping/ Equivalence/ Read-Across Concepts Based on Physical-Chemical Properties (Gera-Pc) for Regulatory Regimes- ENV/JM/MONO(2016)3,” 2016. [Online]. Available: <http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm>.
- [9] ECHA, “Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance,” [Online]. Available: <http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>.

- [10] S. Sørensen and A. Baun, "Controlling silver nanoparticle exposure in algal toxicity testing – A matter of timing," *Nanotoxicology*, vol. 9, no. 2, pp. 201-209, 2015.
- [11] N. Hartmann, K. Jensen, A. Baun, K. Rasmussen, H. Rauscher, R. Tantra, D. Cupi, D. Gilliland, F. Pianella and J.-M. Riego Sintes, "Techniques and protocols for dispersing nanoparticle powders in aqueous media – is there a rationale for harmonization?," *Journal of Toxicology and Environmental Health, Part B Critical Reviews*, vol. 18, no. 6, pp. 299-326, 2015.
- [12] G. Cornelis, "Fate descriptors for engineered nanoparticles: the good, the bad, and the ugly," *Environmental Science: Nano*, vol. 2, pp. 19-26, 2015.
- [13] S. Ottofuelling, F. Von Der Kammer and Hofmann, "Commercial titanium dioxide nanoparticles in both natural and synthetic water: comprehensive multidimensional testing and prediction of aggregation behavior," *Environmental Science and Technology*, vol. 45, p. 10045–10052, 2011.
- [14] D. Cupi, N. Hartmann and A. Baun, "The influence of natural organic matter and aging on suspension stability in guideline toxicity testing of silver, zinc oxide, and titanium dioxide nanoparticles with *Daphnia magna*," *Environmental Toxicology and Chemistry*, vol. 34, no. 3, p. 497–506, 2015.
- [15] OECD, "Test No. 210: Fish, Early-life Stage Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2," 2013. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761.
- [16] C. Smith, B. Shaw and R. Handy, "Toxicity of single-walled carbon nanotubes to rainbow trout, (*Oncorhynchus mykiss*): Respiratory toxicity, organ pathologies, and other physiological effects," *Aquatic Toxicology*, vol. 82, no. 2, pp. 94-109, 2007.
- [17] G. Federici, B. Shaw and R. Handy, "Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress, and other physiological effects," *Aquatic Toxicology*, vol. 84, pp. 415-430, 2007.
- [18] E. Oberdörster, "Manufactured nanomaterials (Fullerenes, C-60) induce oxidative stress in the brain of juvenile largemouth bass," *Environ. Health Perspect.*, vol. 112, no. 10, pp. 1058-1062, 2004.
- [19] X. Zhu, L. Zhu, Y. Lang and Y. Chen, "Oxidative stress and growth inhibition in the freshwater fish *Carassius auratus* induced by chronic exposure to sublethal fullerene aggregates," *Environmental Toxicology and Chemistry*, vol. 27, no. 9, pp. 1979-1985, 2008.
- [20] S. Wong, P. Leung, A. Djurišić and K. Leung, "Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility," *Anal Bioanal Chem*, vol. 396, pp. 609-618, 2010.
- [21] OECD, "Test No. 202: *Daphnia* sp. Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2," 2004. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761.

- [22] OECD, "Test No. 211: Daphnia magna Reproduction Test, OECD Guidelines for the Testing of Chemicals, Section," 2008. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761.
- [23] S. B. Lovern, J. R. Strickler and R. Klaper, "Behavioral and physiological changes in Daphnia magna when exposed to nanoparticle suspensions (titanium dioxide, nano-C60, and C60HxC70Hx)," *Environ Sci Technol*, vol. 4, no. 12, pp. 4465-70, 2007.
- [24] S. Sørensen, R. Hjorth, C. Delgado, Hartmann, N.B and A. Baun, "Nanoparticle ecotoxicity – physical and/or chemical effects?," *Integrated Environmental Assessment and Management*, vol. 11, no. 4, p. 719–728, 2015.
- [25] K. T. Kim, S. J. Klaine, J. Cho, S. H. Kim and S. D. Kim, "Oxidative stress responses of Daphnia magna exposed to TiO₂ nanoparticles according to size fraction," *Science of The Total Environment*, vol. 408, no. 10, pp. 2268-2272, 2010.
- [26] R. Klaper, J. Crago, J. Barr, D. Amdt, K. Setyowati and J. Chen, "Toxicity biomarker expression in daphnids exposed to manufactured nanoparticles: Changes in toxicity with functionalization," *Environmental*, vol. 157, no. 4, pp. 1152-1156, 2009.
- [27] G. P. S. Marcone, A. C. Oliveira, G. Almeida, G. A. Umbuzeiro and W. F. Jardim, "Ecotoxicity of TiO₂ to Daphnia similis under irradiation," *Journal of Hazardous Materials*, Vols. 211-212, pp. 436-442, 2012.
- [28] OECD, "Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD Guidelines for the Testing of Chemicals, Section 2," 2011. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761.
- [29] R. Hjorth, S. Sørensen, M. Olsson, A. Baun and N. Hartmann, "A certain shade of green: Can algal pigments reveal shading effects of nanoparticles?," *Integrated Environmental Assessment and Management*, vol. 12, no. 1, p. 200–202, 2016.
- [30] N. Hartmann, C. Engelbrekt, Zhang, U. J. J, K. Kusk and A. Baun, "The challenges of testing metal and metal oxide nanoparticles in algal bioassays: titanium dioxide and gold nanoparticles as case studies," *Nanotoxicology*, vol. 7, no. 6, p. v, 2013.
- [31] OECD, "Series on the Safety of Manufactured nanomaterials. Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme. First revision". ENV/JM/MONO(2009)20/REV," 2009. [Online]. Available: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2009\)20/rev&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)20/rev&doclanguage=en).
- [32] J. Quik, "Fate of nanoparticles in the aquatic," 2013. [Online]. Available: http://www.ru.nl/publish/pages/664721/phd_thesis_jquik.pdf.
- [33] ECHA, "Appendix R.7.13-2 Guidance on IR&CSA: Environmental risk assessment for metals and metal compounds," [Online]. Available: <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>.
- [34] OECD, "Test No. 225: Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment, OECD Guidelines for the Testing of Chemicals, Section 2," 2007. [Online].

Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761.

- [35] OECD, "Test No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment, OECD Guidelines for the Testing of Chemicals, Section 2," 2004. [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761.
- [36] OECD, "Test No. 219: Sediment-Water Chironomid Toxicity Using Spiked Water, OECD Guidelines for the Testing of Chemicals, Section 2," [Online]. Available: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761.
- [37] N. Hartmann, L. Skjolding, S. Hansen, J. Kjølholt, F. Gottschalck and A. Baun, *Environmental fate and behaviour of nanomaterials- New knowledge on important transformation processes- Environmental Project No. 1594*, The Danish Environmental Protection Agency., 2014.
- [38] B. Allen, G. Kotchey, Y. Chen, N. Yanamala, J. Klein-Seetharaman, V. Kagan and A. Star, "Mechanistic Investigations of Horseradish Peroxidase-Catalyzed Degradation of Single-Walled Carbon Nanotubes," *Journal of the American Chemical Society*, vol. 131, no. 47, p. 17194–17205, 2009.
- [39] K. Schreiner, T. Filley, R. Blanchette, B. Bowen, R. Bolskar, W. Hockaday, C. Masiello and J. Raebiger, "White-Rot Basidiomycete-Mediated Decomposition of C-60 Fullerol," *Environmental Science & Technology*, vol. 43, no. 9, p. 3162–3168, 2009.
- [40] Y. Zhao, A. Allen and A. Star, "Enzymatic degradation of multiwalled carbon nanotubes," *The Journal of Physical Chemistry A*, vol. 115, no. 34, p. 9536–9544, 2011.
- [41] L. Zhang, E. Petersen, M. Habteselassie, L. Mao and H. Q, "Degradation of multiwall carbon nanotubes by bacteria," *Environmental Pollution*, vol. 181, pp. 335-339, 2013.
- [42] R. Tantra, A. Cackett, R. Peck, D. Gohil and J. Snowden, "Measurement of Redox Potential in Nanoecotoxicological Investigations," *Journal of Toxicology*, vol. 2012, pp. Article ID 270651, 7 pages, 2012.
- [43] B. Nowack, J. Ranville, S. Diamond, J. Gallego-Urrea, C. Metcalfe, J. Rose, N. Horne, A. Koelmans and S. Klaine, "Potential scenarios for nanomaterial release and subsequent alteration in the environment," *Environmental Toxicology and Chemistry*, vol. 31, no. 1, pp. 50-9, 2012.
- [44] OECD, "Series on the Safety of Manufactured Nanomaterials- No.63- Physical-chemical parameters: measurements and methods relevant for the regulation of nanomaterials- ENV/JM/MONO(2016)2," 2016. [Online]. Available: <http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm>.
- [45] OECD, "Series on the Safety of Manufactured Nanomaterials. No. 65. Physical-Chemical Properties of Nanomaterials: Evaluation of Methods Applied in the OECD-WPMN Testing Programme," [Online]. Available: <http://www.oecd.org/env/ehs/nanosafety/publications-series-on-safety-of-manufactured-nanomaterials.htm>.

- [46] G. V. Lowry, K. B. Gregory, S. C. Apte and J. R. Lead, "Transformations of Nanomaterials in the Environment," *Environmental Science & Technology*, vol. 46, no. 13, pp. 6893-6899, 2012.
- [47] V. Stone, B. Nowack, A. Baun, N. van den Brink, F. von der Kammer, M. Dusinska, R. Handy, S. Hankin, M. Hasselov, E. Joner and T. Fernandes, "Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterisation," *Science of the Total Environment*, vol. 408, pp. 1745-1754, 2010.
- [48] ECHA, "Guidance on information requirements and chemical safety assessment Chapter R.7b: Endpoint specific guidance," [Online]. Available: <http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>.
- [49] A. Sureshbabu, R. Kurapati, J. Russier, C. Menard-Moyon, I. Bartolini, M. Meneghetti, K. Kostarelos and A. Bianco, "Degradation-by-design: Surface modification with functional substrates that enhance the enzymatic degradation of carbon nanotubes," *Biomaterials*, vol. 72, pp. 20-28, 2015.
- [50] M. Kiser, P. Westerhoff, T. Benn, Y. Wang, J. Perez-Rivera and K. Hristovski, "Titanium nanomaterial removal and release from wastewater treatment plants.," *Environmental Science & Technology*, vol. 43, no. 17, pp. 6757-6763, 2009.
- [51] M. Kiser, J. Ryu, H. Jang, K. Hristovski and P. Westerhoff, "Biosorption of nanoparticles to heterotrophic wastewater biomass," *Water Research*, vol. 44, no. 14, p. 4105-4114, 2010.
- [52] M. Kiser, D. Ladner, K. Hristovski and P. Westerhoff, "Environmental Science & Technology," *Nanomaterial transformation and association with fresh and freeze-dried wastewater activated sludge: implications for testing protocol and environmental fate*, vol. 46, no. 13, p. 7046-7053, 2012.
- [53] B. Pan and B. Xing, "Adsorption mechanisms of organic chemicals on carbon nanotubes," *Environmental Science & Technology*, vol. 42, no. 24, p. 9005-9013, 2008.
- [54] S. Gartiser, F. Flach, C. Nickel, M. Stintz, S. Damme, A. Schaeffer, L. Erdinger and T. Kuhlbusch, "Behavior of nanoscale titanium dioxide in laboratory wastewater treatment plants according to OECD 303 A," *Chemosphere*, vol. 104, pp. 197-204, 2014.
- [55] A. Jemec, A. Kahru, A. Potthoff, D. Drobne, M. Heinlaan, S. Böhme, M. Geppert, S. Novak, K. Schirmer, R. Rekulapally, Singh, V. Aruoja, M. Sihtmäe, K. Juganson, A. Käkinen and D. Kühnel, "An interlaboratory comparison of nanosilver characterisation and hazard identification: Harmonising techniques for high quality data," *Environment International*, vol. 87, p. 20-32, 2016.
- [56] OECD, "Series on Testing and Assessment. No. 29. Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media. ENV/JM/MONO(2001)9," 2001. [Online]. Available: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2001\)9&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2001)9&doclanguage=en).

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