Organisation for Economic Co-operation and Development

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Guidance Document for the testing and interpretation of data on dissolution rate and dispersion stability of nanomaterials for effects and exposure assessment

1st DRAFT for WNT Comments (JULY2019)

This is the first draft to be circulated for WNT comments. It includes comments provided during the Expert commenting round that took place (April-May 2019).

The revised draft, clean and tracked changes version, together with responses to comments received have been made available in the restricted site.

The WNT is invited to review this draft and provide comments by **19th September 2019.**

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1 Introduction

1. In the OECD Expert Meeting hosted by Germany in Berlin 2013 (OECD 2014a), it was identified that dissolution rate and dispersion stability in the environment are important parameters for nanomaterials and that Test Guidelines (TGs) should be developed for these parameters. As these parameters are often interlinked it was also acknowledged that an overarching guidance document (GD) would be beneficial as well.

2. TG 318 (OECD 2017) on dispersion stability of manufactured nanomaterials in simulated environmental media was published in 2017.

3. The development of a TG on dissolution rate in environmental media was included as OECD WNT project 3.10 in the Test Guideline Programme workplan in 2014 (latest version OECD 2018), but could not be finished so far. In the meantime, also other relevant methods for dissolution rate testing in water and biological fluids and transformation in the environment (WNT project 1.5 and WNT project 3.16) were included to the WNT work plan. Therefore, to make progress it was concluded to build the dissolution relevant content for the overarching GD on the available information, including the current draft documents of WNT project 3.10, scientific literature, and GD 29 (OECD 2001). For the short-term, it provides guidance on experimental steps and procedures of batch and dynamic flow-through methods and decision support when to use them for nanomaterials (Chapter 2). After mentioned WNT processes are finalised and TGs available, an update of this GD might be needed.

4. This GD provides guidance for the methods to address above mentioned endpoints, in particular on the influence of various experimental conditions on the performance and outcomes of those methods. In addition, this GD aims to give support for the interpretation of results.

5. In **Chapter 2** provides guidance for the determination of solubility and dissolution rate based on batch test and flow-through methods as well as on how to evaluate and report the gained test results. .

6. Specific guidance on TG 318 is given in **Chapter 3** including further experimental conditions than described in the TG, guidance to account for heteroagglomeration (section 3.3), and deriving attachment coefficient(s) (section 3.4). In order to address the latter issues, the state of the knowledge was included from available scientific literature. Furthermore, guidance is provided on the interpretation and presentation of data addressing the endpoint.

7. The use of data generated by dissolution testing and testing of dispersion stability using TG 318 for possible further nano-specific fate and effect testing and assessment strategies is presented in **Chapter 4.** A testing strategy is presented in section 4.2. Furthermore, mutual influence of the two endpoints to each other is discussed, i.e. dispersion stability will influence dissolution rate and *vice versa.*

8. In **Chapter 5**, this GD provides recommendations on the use of output data from dissolution rate and dispersion stability tests to derive input parameters for exposure models.

9. **Chapter 6** provides information on the use of this GD in relation to other OECD TGs and GDs, including the foreseen GD on aquatic and sediment toxicity testing (see

section 1) and the foreseen GD on the apparent accumulation potential of nanomaterials in fish (see section 6.3). As fate estimations of nanomaterials in soil and sediment are challenging to conduct, the GD also gives advice on screening possibilities for dispersion stability and dissolution rates by variating the environmental conditions to mimic those in soil and sediment (see section 6.2), and this is linked to the foreseen nano-specific GD for OECD TG 312.

2 Testing of solubility and dissolution rate

10. It is important to clearly distinguish between the terms solubility, dissolution and dissolution rate (see Annex 1 for definitions). Solubility and dissolution rate of nanomaterials are important to predict their fate and behaviour in the environment and for understanding the changes in their bioavailability, reactivity, fate, and toxicity. Dissolution rates from nanomaterials are particularly important in determining risk/hazard since the rate of release of ions/molecules prior to interaction/complexation with ligands may be more important than equilibrium concentrations.

11. General methods for the determination of solubility and dissolution rate are available (e.g. OECD TG 105 (OECD1995), Superb et al. 2012, ISO 19057 (ISO 2017) having different advantages and disadvantages. So far, no specific OECD TG is available for determination of solubility and dissolution rate for nanomaterials. However, there are two WNT projects ongoing (WNT project 3.10 "TG on Dissolution Rate of Nanomaterials in Aquatic Environment" and WNT project 1.5 on "Determination of Solubility and Dissolution Rate of Nanomaterials in Water and Relevant Synthetic Biological Media") aiming to provide harmonised approaches for testing solubility and dissolution rate of nanomaterials via static batch testing and dynamic flow-through methods, respectively.

12. In OECD TG 105 (OECD 1995) two methods are described for the determination of solubility of substances, a static batch test and a dynamic test. For determining dissolution (rate) of nanomaterials the same set-up may be used with some modifications. In addition, the existing OECD GD 29 (OECD 2001) for metals and metal compounds may be applicable to some nanomaterials. It describes a similar batch test as the one developed in WNT project 3.10. Scientific basis for the flow-through method currently foreseen in the WNT project 1.5 can be found in literature e.g. in Koltermann-Juelly et al. 2018.

2.1. Static Batch Test

13. Currently a draft TG on "Determining the Dissolution of Metal Nanomaterials in Aquatic Media" is in preparation (WNT project 3.10) but not yet available. This draft TG is based on OECD GD 29 (OECD 2001, Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media) while making some nanospecific amendments. Care should be taken regarding the applicability of OECD GD 29 when considering the purpose of testing solubility and dissolution rate of nanomaterials, respectively. OECD GD 29 aims to provide supplemental information for aquatic ecotoxicity testing of metal and metal compounds. As indicated in OECD GD 29 "The intent of the screening test, performed at a single loading, is to identify those compounds which undergo either dissolution or rapid transformation such that their ecotoxicity potential is indistinguishable from soluble forms". As such the original purpose of OECD GD 29 is not to provide a harmonised approach to determine solubility or dissolution rate or to provide information on environmental fate. Some of the pros and cons of a static batch test as described in GD 29 are discussed in this section.

14. Furthermore, several considerations in GD 29 differ from the rationale of testing nanomaterials. For example, while GD 29 asks for testing the smallest available particle of a metal or metal compounds, for nanomaterial the question arises about the solubility or dissolution rate of the specific nanomaterial under investigation. As another example, the

screening test in GD 29 requires test conditions where a metal/metal compound shows highest solubility, whereas for nanomaterials the specific solubility/dissolution rate under certain conditions is of interest (i.e. not necessarily the highest solubility).

15. For testing of solubility and dissolution rate the use of the 24 h screening test of GD 29 can still be of interest and can be in principle be applied, depending on the nanomaterial properties. This seems to work at least for silver nanoparticles (Wasmuth et al 2016). For instance, the screening test can provide solubility limit for all nanomaterials and estimation of dissolution rate for sparingly soluble and slowly dissolving nanomaterials. However, for determining dissolution rate solubility concentration needs to be measured against time with narrow measuring points as long as steady state concentration is not reached.

16. GD 29 prescribes a 0.20 µm filtration method for separating dissolved and non-dissolved compounds. This is not appropriate for nanomaterials that have individual particles or aggregates that are usually smaller than this size cut-off, so further separation is needed.

17. For the screening test the OECD GD 29 proposes the use of reconstituted standard water with pH range between 6 to 8.5 (i.e. water of known composition, for details on media composition see OECD GD 29). However, in principle and based on the purpose of the testing, the 24 h screening test can be also performed under different media conditions (e.g. ecotoxicity media, natural water, simulated media according to TG 318). In any way it is essential to characterise and report test media characteristics as detailed as possible (e.g. pH, ionic strength should be reported), as media composition considerable influences nanomaterial’s dissolution.

18. When performing the dissolution test as a batch test, it should be realised that derived dissolution rates according to GD 29 may not reflect the dissolution rate under environmental realistic conditions. The ion concentration in the test media may increase, and the solubility limit of an investigated nanomaterial in the specific test medium may be reached before complete dissolution. Longer observation periods will then reduce the apparent dissolution rate. If not considered in the process of deriving the rate constant and the applied model, the obtained value will be depending on the a) solute concentration already present at the start and b) the solute concentration build-up during the experiment. Derivation of dissolution rate of dissolving nanomaterials is not possible for those nanomaterials that show such a rapid increase of the ion concentration in the test media at the beginning of the testing that results in a too steep slope of the dissolution function for time resolved measurements.

19. Three main methods exist in the literature to separate nanomaterials and their aggregates from their dissolved fraction: ultra-centrifugation, dialysis and centrifugal ultrafiltration. Nevertheless, not all of them are recommended for use in the determination of dissolution rates.

20. Ultra-centrifugation is not recommended for several reasons. Firstly, it is difficult to calculate the optimal centrifugal settings (speed and time) to guarantee complete centrifugation of nanomaterials, especially for the case of non-spherical particles and when the rotor has no swing-bucket design but is e.g. a fixed angle rotor. Secondly, theoretical optimal centrifugal times are often long relative to the dissolution rate, especially in the case of relatively small nanomaterials. Small nanomaterials need proportionally long centrifugation times to be separated from their dissolved ions and at the same time dissolve faster compared to larger nanomaterials of the same composition. A too slow separation

technique thus hampers the determination of relatively fast dissolution rates. Finally, back-diffusion of centrifuged nanomaterials into the centrifuge vial is likely and may cause artefacts of overestimating dissolution. This is in particular relevant in case sampling for dissolved substance measurement takes place too deep in the centrifugal vial. Thus, both dissolved substances and back-diffused nanomaterials are sampled.

21. Dialysis is also not recommended for separating nanomaterials and their dissolved substances. In this technique, nanomaterials are suspended in a medium within a dialysis bag. Dissolved substances thus need to diffuse through the dialysis membrane into a second compartment where they can be sampled for quantification of the dissolution rate. This process may again be too slow compared to the dissolution process itself (see e.g. Franklin *et al.*, 2000).

22. Centrifugal ultrafiltration is the recommend method for separating nanomaterials and their dissolved substances. Here, a mixture of nanomaterials and their dissolved substances are injected in centrifugal ultrafiltration devices. During centrifugation, nanomaterials and dissolved substances and the test medium are transported towards an ultrafiltration membrane through which nanomaterials cannot pass while their dissolved substances can. The dissolved substances can then be measured in the filtered media.

23. The pore diameter of ultrafiltration is expressed in terms of molecular weight cut-off (MWCO), i.e. the molecular weight of different molecules in the filtration process (usually dextran or polyethylene glycol) that are retained for 90 % by the membrane (Ren et al., 2006). There is also a pore size distribution, rather than a single pore size. The maximum pore diameter of 10 kDa membranes, for instance, is 4.57 nm (Ren et al., 2006). To ensure complete separation between ionic and particulate phases, a MWCO of maximum 3 kDa is recommended.

24. The centrifugal speed and time required to drive a sufficient amount of aqueous solution containing dissolved species to cross the membrane depends on the MWCO and hydrophobicity of the membrane, as well as the chemistry of the medium. The centrifugal settings should be optimized to achieve a filtrate volume sufficient for subsequent measurement. Prewashing of the filter membrane by centrifuging ultrapure water through the membrane is prerequisite for any filtration step to remove dissolved chemicals that could influence the dissolution process. Modifying or pre-treating the membranes can be used if significant issues are observed (Cornelis et al. 2010).

25. In principle the batch test procedure may also be applicable for testing non-metal nanomaterials, but the current analytical possibilities are still limiting these options.

2.2. Dynamic testing of dissolution rates

26. The WNT project 1.5 aims to include two different methods: both a static batch test, and dynamic dissolution testing by a flow-through system. The method applied for a flow-through dissolution test is based on the amended Continuous Flow System mentioned in ISO TR 19057 (ISO 2017). Here, simulated media is continuously pumped from a reservoir through a cell containing a nanomaterials sample. After the media has passed the nanomaterial sample the solute concentration in the fluid can be measured. The method was applied in the past to determine the bio-durability for mineral fibre and its applicability to nanomaterials was presented by Koltermann-Juelly (2018) for the dissolution of 24 (nano)forms of 6 substances (figure 1 below) for various human lung fluids and by Bove et al. (2018) for various gastro fluids.

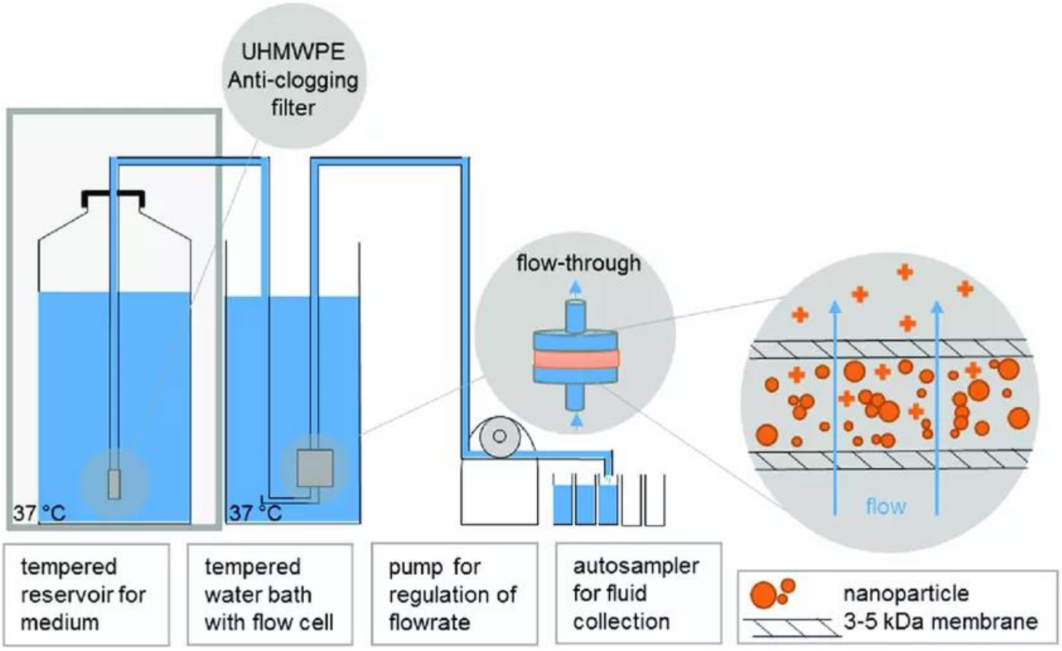

Figure 1. The experimental setup for flow-through testing (from Koltermann-Juelly et al. (2018)). (UHMWPE = Ultra High Molecular Weight Polyethylene)

27. In principle that method can be adapted also to measure dissolution rate in environmental media. However, depending on the purpose test conditions might need to be adapted for difference in environmental compared to biological media. Apart from differences in test media composition, considerations for adaptation include applied test concentration, flow rates and test duration. For instance, for the determination of dissolution rate under environmental relevant conditions considerably different concentrations (e.g. media composition, temperature) and flow rates (first suggestion µg and 1 ml/min) should be used than those used for the dissolution testing in biological media (mg and 2 ml/h), thus saturation effects will hardly occur.

28. The dynamic dissolution test should mimic the condition in a natural water, where the nanomaterial is highly diluted and freely diffusing. The dissolution rate of intermediate and highly soluble materials is among other parameters controlled by the thickness of the boundary layer and the concentration gradient of dissolved ions in the boundary layer. For those nanomaterials the transport of ions away from the particle surface is a limiting factor. The flow rate in the experiment should ideally mimic those conditions. With 2 mL/h saturation effects have been observed with $BaSO_4$, at 1 mL/min these effects are reduced but not totally prevented. Flow rate and test conditions can be in principle modify to mimic other specific environmental relevant conditions.

29. With current scientific knowledge and the co-dependence of the dissolution rate on solubility, thickness of boundary layer and specific surface area/particle size, exact cut off values for applying dynamic dissolution test cannot be given. From current experiences the dynamic test would be suggested if the solubility of the nanomaterial is between 0.1 and 10 mg/L. This range can of course be broadened when test conditions as flow rate and amount of material in the test are adapted to the solubility of the material.

30. In addition, the test procedure can be adapted in such a way that the application of the investigated nanomaterial can be injected directly as a dispersion into the system at

a location between pump and a filter membrane. It is recommended to use a filter membrane of 3 kDa. The schematic figure representing the dynamic test system is presented in figure 2.

31. Similar to the batch test, in principle the flow-through test procedure may also be applicable for testing of non-metal nanomaterials, but the analytical possibilities are still limiting these options.

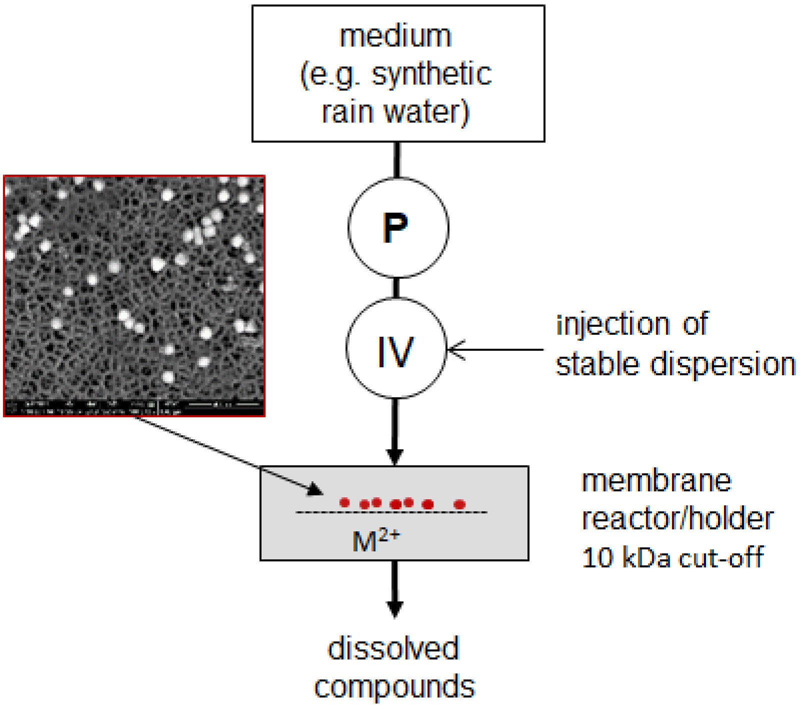

Figure 2. A draft scheme for dynamic testing of dissolution rate (von Kammer 2018, personal communication, to be published soon). (P = pump, IV = Injection Valve).

2.3. Data evaluation and reporting from dissolution testing

32. The concentration of the dissolved fraction should be measured during the test and plotted versus time.

33. The result of the solubility test has to be expressed as mg/L of the ions formed as a consequence of dissolution. As the solubility depends on the starting concentration, also the starting concentration has to be reported. Expression solubility in % is discouraged as this is difficult to interpret and to compare with other data.

34. For most nanomaterials, dissolution follows a first order kinetics. As a result, the dissolution rate will not only depend on the dissolution rate constant and specific surface area, but also on mass. The relationship is:

$$\text{Dissolution rate } = \frac{d\text{m}}{d\text{t}} = -k_{\text{diss}} \cdot \text{m}$$

where m is mass, t is time, and k_{diss} is dissolution rate constant. Dissolution rate can be also expressed as:

$$\text{Dissolution rate } = \frac{d\mathrm{m}}{d\mathrm{t}} = \left(\mathrm{D} \cdot \frac{\mathrm{A}}{\mathrm{h}}\right) \cdot (\mathrm{c_S} - \mathrm{c})$$

where D is the diffusion coefficient of the dissolved species in the medium, A is the surface area of the nanomaterial, h is the thickness of the diffusion layer, c_S is the saturation concentration, and c is the starting concentration. The thickness of the diffusion layer will be dependent on the test condition e.g. if the nanomaterial is agitated during the test performance.

35. Based on this dissolution rate of first order kinetics the dissolution halftime (when half of the nanomaterial is left and half is dissolved, respectively) can be estimated (for information on calculation see e.g. ISO 2017).

36. Results on solubility and dissolution rate have to be reported together with test conditions like media composition, temperature, and test duration.

3 Testing of dispersion stability with TG 318

37. OECD TG 318 (OECD 2017) describes a method for determining the dispersibility and dispersion stability of nanomaterials in aqueous media of different, yet environmentally relevant hydrochemistry. Prerequisite for the experimental approach was to enable the investigation of the dispersion behaviour in a small number of relatively simple tests within a time frame suitable for standard laboratory routine. The hydrochemical conditions in the tests cover those parameters and parameter ranges which are (a) representative for natural waters and (b) are recognized drivers for agglomeration of nanomaterials within concentration range of the TG (Monikh et al. 2018). Hence the composition of the test media resembles only those compounds in surface water that are relevant for the agglomeration process and exist in a concentration range where they can become relevant.

38. Dispersion stability as measured using TG 318 actually determines homoagglomeration (attachment of nanomaterial to each other) under consideration of environmental parameters which have a major influence on the dispersion stability of nanomaterials over a fixed time-span of 6 hours. This enables a direct comparison of nanomaterials with each other and how they will behave in test systems. For comparison with media which differ in composition from the test media in TG 318, the agglomeration-relevant compounds in the media should be compared. These are the concentrations of divalent cations and anions, the pH, the concentration of natural organic matter.

39. The kinetics of the homoagglomeration processes are depending on the number concentration of the nanomaterials and the progression of agglomeration. To be able to directly compare results among different nanomaterials and also to finish the test over a period of 6 hours, the starting concentration must be set to a fixed particle number concentration. In this way the agglomeration process is almost independent of particle size and density. Comparisons have shown that the starting concentration in particle number should not vary more than one order of magnitude between different nanomaterials (i.e. roughly plus or minus half an order of magnitude). To obtain the required mass concentration of the nanomaterial, the mass concentration of the nanomaterial in the stock dispersion has to be converted into particle number concentration by using the average particle diameter and material density as described in TG 318. It is acknowledged that, especially for broad distributed nanomaterials, the average particle size will not convert correctly into the particle number concentration, however, the influence on the test outcome appears to be small (order of magnitude accuracy required) so that the additional effort for precisely determining the particle size distribution and considering it in the number calculation appears not necessary. However, if precise data on the particle size distribution are available, it is advised to use this information with broad distributed samples. An example is presented in figure 3, where experimental data over the test period of 6 hours for TiO_2 NM105 nanomaterial under stable (0 mM $Ca(NO_3)_2$) and destabilizing (5 mM $Ca(NO_3)_2$) conditions and with two different starting particle number concentrations are shown.

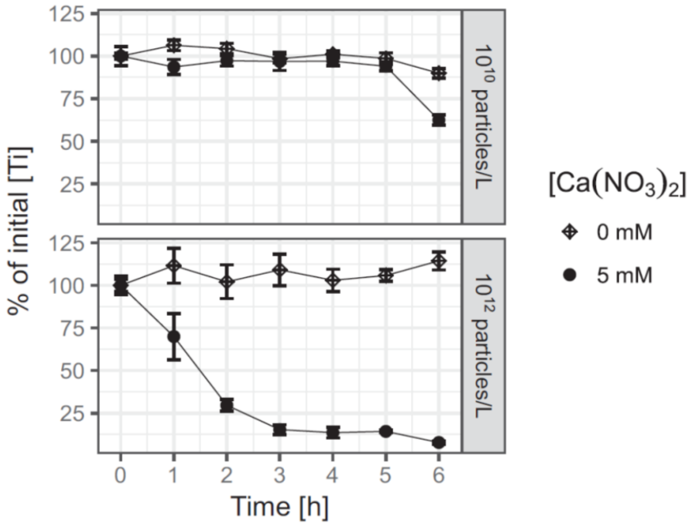

Figure 3. Experimental data over the test period of 6 h for TiO_2 NM105 nanomaterials under stable (0 mM $Ca(NO_3)_2$) and destabilizing (5 mM $Ca(NO_3)_2$) conditions and two different starting particle number concentrations (Monikh et al. 2018).

40. With progressing agglomeration and sedimentation of the agglomerates, the concentration in the supernatant will decrease over time. The sedimentation velocity, and with this also the rate of decrease of nanomaterial, in the supernatant is depending on many factors, e.g. the density of the primary particles, the apparent density of the formed agglomerate, the structure of the agglomerate, the surface chemistry, and how the water flows around or through the agglomerate. To eliminate at least the effect of density, the last step after 6 hours is a centrifugation step where the run conditions of the centrifugation are set to achieve a size cut-off at > 1 µm. TG 318 describes how to calculate the centrifugation conditions. After the centrifugation step, the remaining concentration of the nanomaterials in the supernatant of the dispersion is analysed. The centrifugation step after 6 hours is best suited to compare different materials with each other, while the hourly measurements between 1 to 5 h show the behaviour of the material in a water column.

41. Apart from the intrinsic properties of the nanomaterial, the composition of the medium is the driver for the stabilisation or destabilisation of the dispersions. Therefore, the test considers the concentrations of electrolytes that are dominating this process and sufficiently abundant in natural waters to become relevant in the process, i.e. divalent ions, natural dissolved organic matter and pH. The concentrations of these compounds were set to represent about 95% of the conditions found in natural waters. See figure 4 for visualization of effects of different experimental conditions and various effects of different components on dispersion stability in Table 1.

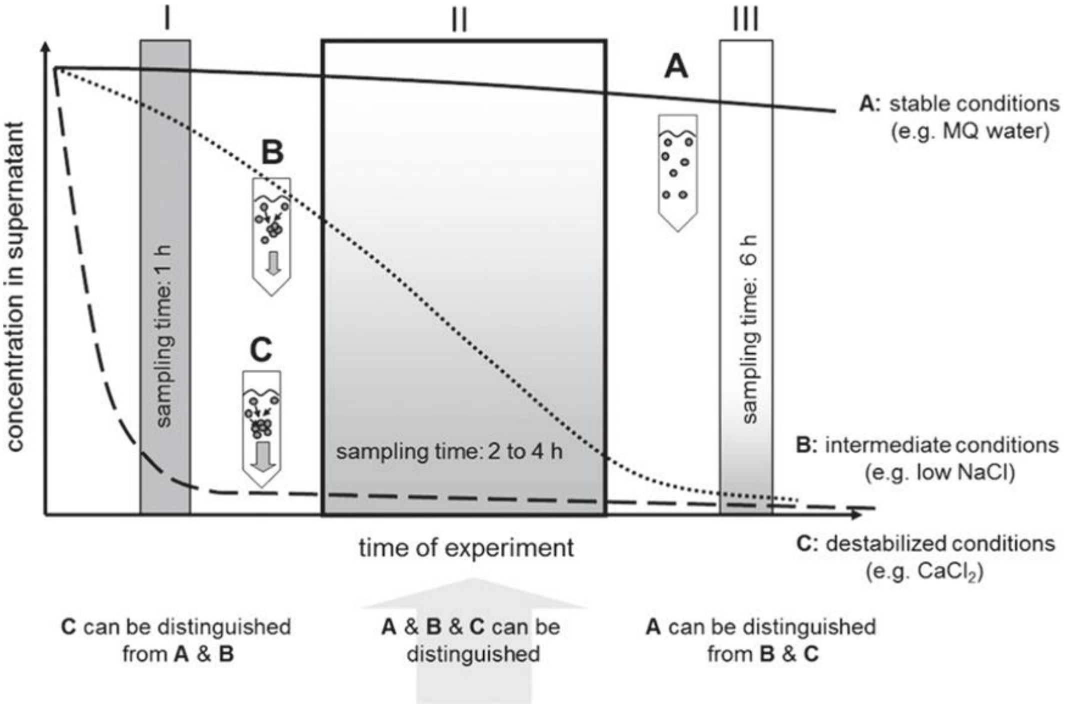

Figure 4. A general visualization of effect in different experimental conditions and how this can be used to differentiate between suspensions of A high, B intermediate and C low stability. (Monikh et al. 2018)

Table 1. Role and effect of the selected components in the synthetic water (Monikh et al. 2018)

compound	represents	effect	effect strength	abundance
Na^+	monovalent cations not included	destabilisation	low	medium
Ca^{2+}	divalent cations	destabilisation stabilisation when adsorbing to surfaces of positively charged materials	medium	high
Mg^{2+}	divalent cations less complexed by NOM	destabilisation relevant when NOM is high and complexes Ca^{2+}	low	low
NO_3^-	monovalent anions	destabilisation non-adsorbing non-complexing	low	low
SO_4^{2-}	divalent cations	destabilisation, especially positively charged particles	medium	medium
Al^{3+}	trivalent cations not included	destabilisation	high	very low
SR-NOM	dissolved organic matter	stabilisation through surface adsorption with electrostatic (negative) and steric effect charge reversal (positive to negative) through complexation of destabilising cations destabilising when adsorption to positive surfaces reduces net charge of surfaces	high	medium

42. A decision tree is presented in the TG 318 to determine whether the nanomaterial of interest requires only a screening procedure or if an in-depth testing has to be performed. The decision tree allows nanomaterials to be categorised as generally stable dispersible under all test conditions, non-dispersible or dispersible depending on the hydrochemical

conditions. The screening test is performed in the presence of natural organic matter, which will stabilise most nanomaterials against agglomeration. This will put many nanomaterials into the category “dispersible, no detailed testing in TG 318 necessary”.

43. The NOM added to the test vial has three roles in the test: (a) it acts as a pH buffer stabilizing the adjusted pH, especially at pH 9, (b) it complexes Ca^{2+} and reduces thereby the activity of the destabilising Ca^{2+} ion, (c) it adsorbs to the surface of the nanomaterial and adds to the negative charge density, thereby reducing the net positive charge that can lead to destabilisation. If present in sufficient amounts, it eventually may reverse the charge to negative and can increase the magnitude of the negative charge density. The amount of natural organic matter to be used is standardized to 10 mg/L DOC in 40 mL (400 µg DOC) and a calculation tool for the required minimum DOC is provided in the TG318.

44. Applicability of TG 318 for different nanomaterial types should be considered based on the available data on similar or resembling nanomaterials, e.g. based on their shape and size (spherical, rod, platelets, fibre-like). Three considerations are important to judge a priori if the test can be applied to a nanomaterial: (a) the nanomaterials density must be > 1 g/cm^3, (b) the mass concentration calculated for the required particle number concentration must be at least 10 times above the detection limit of the analytical method, (c) an analytical method with sufficient sensitivity for the nanomaterial or an equally distributed component is required. The requirement (b) stems from the consideration that even with 90% removal from the water column the nanomaterial should still be quantifiable in the supernatant. Especially for small, low density particles, the mass concentration originating from the recommended number concentration might challenge the ICP-OES based methods and at some point also routinely operated ICP-MS methods. The required detection limit (10% of initial concentration) for a SiO_2-NP of 15 nm would be ~0.2 µg/L (Si).

45. Regarding the analytical approach to quantify the nanomaterials in the supernatant in principle any method/instrument can be applied which offers the required detection limits for the nanomaterial or a component of it.

3.1. Data presentation and evaluation for TG 318

46. When the dispersion stability of a nanomaterial is tested according to TG 318 either the screening test is sufficient or a full testing is required.

47. There are various ways to present the retrieved data for the screening test or to visualize the influence of electrolyte concentration, pH, and presence of NOM. Illustrative ways of data presentation from dispersion stability studies using TG 318 can be found in figure 5, 6 and 7. The spreadsheet tool [1]together with this GD can help to visualize the data in a more harmonised format

48. Figure 5 shows a schematic example on dispersion stability for results of the screening test with the three possible outcomes according to the TG 318 decision tree.

49. In figure 6 the output of the tool for illustrating the full tests’ results (following Monikh et al. 2018) is presented. Here the results are presented in a three-dimensional matrix considering various hydrochemical conditions and the resulting dispersion stability. Higher colloidal stability (less agglomeration/sedimentation) is reflected by a higher

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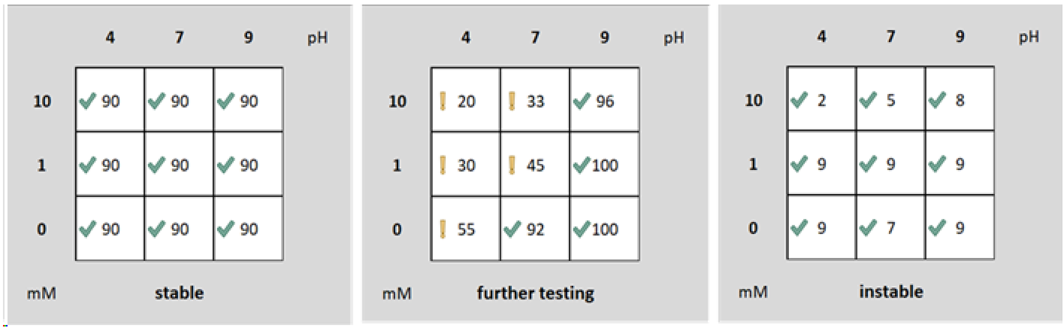
[1] The spreadsheet has been made available as a separate Excel document.

remaining concentration (brighter shade in the plots) in the dispersion relative to the starting concentration (0-100%).

50. By means of the full test beside dispersion stability also information on the underlying processes leading to the removal from the water column can be elucidated. Figure 7 presents an example of a dispersion stability plot of the full test for one test condition where the dispersion stability measured at each hour is plotted over the time. Possible interpretation of removal processes based on the removal function are shown in Figure 8.

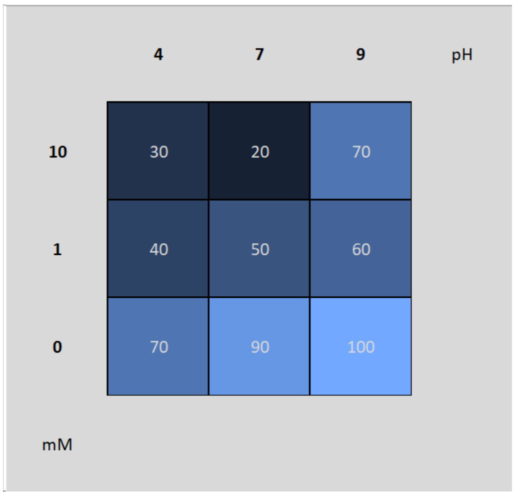


	4	7	9	pH
10	✓ 90	✓ 90	✓ 90	
1	✓ 90	✓ 90	✓ 90	
0	✓ 90	✓ 90	✓ 90	
mM		**stable**		

	4	7	9	pH
10	! 20	! 33	✓ 96	
1	! 30	! 45	✓100	
0	! 55	✓ 92	✓100	
mM		**further testing**		

	4	7	9	pH
10	✓ 2	✓ 5	✓ 8	
1	✓ 9	✓ 9	✓ 9	
0	✓ 9	✓ 7	✓ 9	
mM		**instable**		

Figure 5. Output from the spreadsheet tool for the screening test (The numbers in the boxes indicate the % of initial concentration left in the supernatant at the end of the test). Left panel: all tests are completed with ≥90 % of the nominal (initial) concentration left in the supernatant after 6 h (indicating high stability). Middle panel: some conditions lead to stable (≥90 %), some to intermediate stability (≥10 % and ≤ 90%), further testing is required in a full test. Right panel: all conditions lead to ≥ 90 % sedimentation from the water column (≤10 % stability), indicating an unstable nanomaterial under tested conditions.



	4	7	9	pH
10	30	20	70	
1	40	50	60	
0	70	90	100	
mM				

Figure 6. Dispersion stability regarding different environmental conditions (electrolytes, pH). Higher stability (less agglomeration/sedimentation) is reflected by a higher remaining concentration (brighter shade in the plots) in the dispersion relative to the starting concentration (0-100%). The numbers in the boxes represent the remaining percentage of the nanomaterial in the dispersion, thus the dispersion stability in %. (from Monikh et al. 2018).

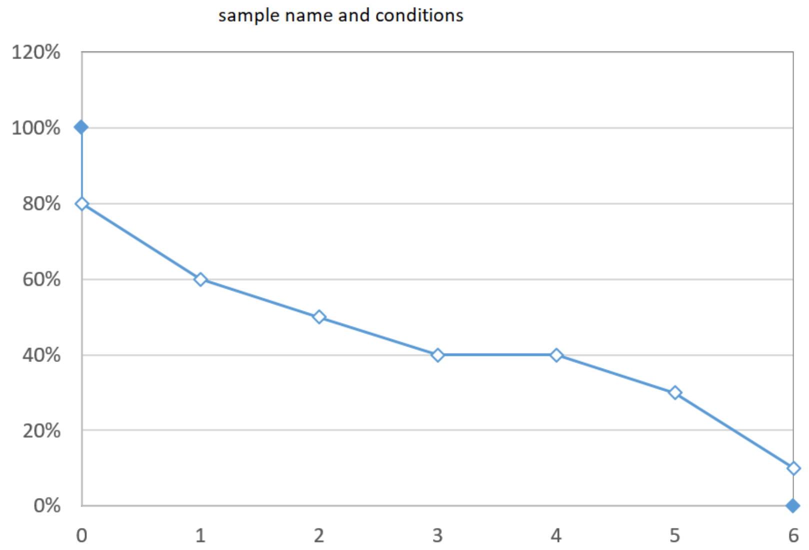


Figure 7. An example of dispersion stability plot of the full test for one (here not further specified) test condition.

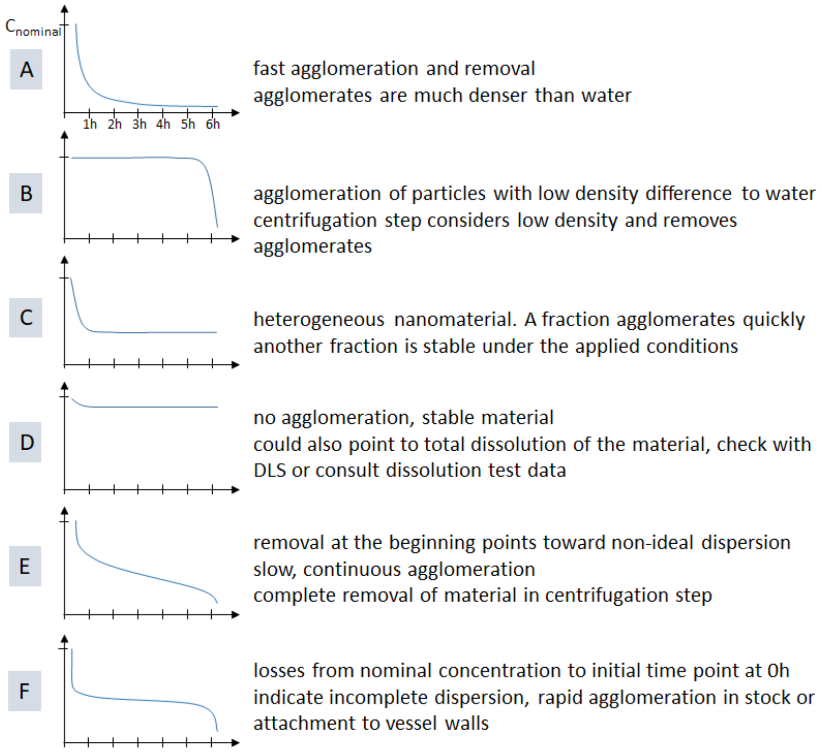


Figure 8. Possible shapes of the removal function over time and suggested interpretations.

3.2. Alternative test conditions

51. TG 318 covers only synthetic waters that resemble the bandwidth of agglomeration-relevant components found in surface waters. In principle TG 318 can also be performed with other media than those used in TG 318, e.g. ecotoxicological test media. When using any other test media it should be assured that the addition of the nanomaterial to the medium does not significantly change media conditions, e.g. the pH. A good estimate can be drawn from results of TG 318 with synthetic waters if the agglomeration-relevant components of the test media (sum of divalent cations, sulphate) are comparable with TG 318 conditions (the full test without NOM). When using alternative test conditions compared to TG 318 it is of utmost importance to characterise and report the test media compositions and conditions and also to compare those with test media compositions and conditions of TG 318 for the data evaluation and interpretation. This will also enable retrospective analysis of studies compared with new data produced in the future.

52. TG 318 can be used with natural waters to investigate the dispersion stability and agglomeration behaviour in these waters. To prevent a situation where homo- and heteroagglomeration takes place in an uncontrolled way, the water sample should be filtered over a filter membrane with pore size equal or smaller than 0.1 µm or subjected to ultracentrifugation to remove all sorts of natural particles, microorganisms, µm-sized debris, colloids and nanoparticles from the sample. It should also be taken into account that the obtained result is a (very precise) descriptive value for this one sample only, resembling a unique and constantly changing situation regarding hydrochemical composition and type and concentration of NOM. Whether it is possible to transfer this one result to the sampled surface waterbody in general depends on the spatial and temporal variability of the waterbody. If the unfiltered sample shall be tested with TG 318 the presence of natural suspended particulate matter will make it necessary to apply the variant of TG 318 that deals with heteroagglomeration (see section 3.3).

53. The transferability of the data from TG 318 studies to higher tier testing (e.g. ecotoxicological studies) should be carefully evaluated as the used simulated environmental media differs from ecotoxicological test media which aims to promote animal vitality rather than to mimic natural habitats. When possible, it would be beneficial to test the dispersion stability with TG 318 using the test media used in the ecotoxicological test as recommended in the OECD draft GD for Aquatic (and Sediment) Toxicological Testing of Nanomaterials (WNT project 2.51). However, in many ecotoxicological tests, even in an algal test (TG 201), test media composition during the 72 hours test will change by algal exudates and photosynthesis. This will result in pH shift, various ionic compositions and different NOM characteristics that will result in changes in dispersion stability.

54. NOM is a natural product with an enormous variability in structure, molecular weight distribution, conformation, composition and purity. The type and quality of the used NOM or even NOM in natural waters or test media with intrinsic NOM will have effects on dispersion stability. This should be taken into account for data evaluation and in estimations for environmental conditions. One has to distinguish between processed commercial products resembling NOM or unprocessed natural NOM as part of a natural water sample. The commercial products are more or less close to reality regarding their properties and are more likely to enable repeatability of the results. In contrast, natural NOM might trigger a behaviour of the nanomaterial that is linked to the composition of the NOM, which might be very unique in time and space and not fully resemble the surface water the NOM was sampled from. In TG 318 2R101N Suwannee River NOM (SRNOM)

is recommended as standardised and purified material. Due to differences in the composition of NOM from different sources, it is difficult to compare results obtained by the use of different types of NOM. Therefore, the alternatively used NOM should be characterised as much as possible, at least the minimum DOC content after properly cleaned from ions and ash should be determined. DOC content and treatment should be reported together with the test results. It is advisable to always test the nanomaterial according to the conditions presented in TG 318 in order to obtain comparative "benchmark" data.

3.3. Testing of heteroagglomeration

55. The heteroagglomeration of nanomaterials (mainly engineered nanoparticles, referred to as ENPs) with suspended particulate matter (SPM), which is ubiquitous in natural surface waters, is a crucial process affecting the environmental transport and fate of nanomaterials (Praetorius et al. 2012, Quik et al. 2014, Therezien 2014, Gao and Lowry 2018).

56. The need for consideration of heteroagglomeration as an aspect of nanomaterial fate has been discussed already during the development of TG 318 on dispersion stability (Baun et al. 2017). Due to the complexity and diversity of natural SPM and the possible interaction mechanisms of nanomaterials with it, it seems impossible to decide on a representative set of SPMs for standardisation on one hand, while on the other hand a restricted number of SPMs could lead to disproportional uncertainty in the estimation of (hetero)attachment efficiencies (see section 3.4). Furthermore, methods are not yet progressed to develop a fully validated TG for heteroagglomeration testing.

57. Many studies report measurements of different endpoints reflecting heteroagglomeration behaviour (e.g. agglomeration rate, sedimentation, attachment efficiency) with diverse nanomaterials under various conditions (e.g. Praetorius et al 2014, Labille et al 2015, Velzeboer et al. 2014, Huynh et al 2012, Quik et al. 2014, Geitner 2017, Barton et al. 2014).

58. TG 318 is in principle fit for purpose to also investigate heteroagglomeration and roughly estimate the attachment efficiency (α_{hetero} values). An important question of interest is: What is the time frame (seconds-hours, days-weeks, months or more) we need to consider until the majority of free nanomaterials has turned into SPM-attached nanomaterials and therewith their transport regime (SPM attached nanomaterials are transported like SPM) or bioavailability might be changed? In most cases an orders-of-magnitude based category of α_{hetero} (e.g. "low", "medium" and "high") will be sufficient to address this question. α_{hetero} can be used as an indicator for the expected half-life ($t_{1/2}$) of free nanomaterials under certain conditions (e.g. SPM concentrations). An example for CeO_2 is shown in table 2. In multimedia fate modelling a similar regime of sensitivity to α_{hetero} for predicted environment concentrations is observed (Meesters et al. 2019). Thus, based on the available scientific knowledge and methods, guidance for heteroagglomeration testing and presentation of data can be provided. Necessary considerations and modifications of the test setup include the following issues presented below.

Table 2: Relationship of attachment efficiency (αhetero) and expected half-life (t1/2) of free nanomaterials (5ppb CeO_2, d = 25nm) in presence of 1-150ppm SPM.

heteroaggregation attachment efficiency	expected free ENP (5 ppb CeO_2, d =25 nm) half-life range for 150 ppm – 1 ppm SPM (d_n =1.5 µm, ρ =1.5 g/cm³)
$\alpha_{hetero} \approx 0.1 - 1$	$t_{1/2} \approx$ seconds – 1 day
$\alpha_{hetero} \approx 0.01$	$t_{1/2} \approx$ hours – days or (few) week(s)
$\alpha_{hetero} \approx 0.001$	$t_{1/2} \approx$ day(s) – month(s)
$\alpha_{hetero} \approx 0.0001$	$t_{1/2} \approx$ week(s) – (few) year(s)

59. The hydrochemical background conditions suggested in TG 318 with regards to electrolyte compositions and concentrations can be equally applied to heteroagglomeration, as the mechanistic principles are the same. It is however suggested to use the “alternative medium” including SO_4^{2-} to cover the effects of divalent anions on agglomeration, especially if one of the components (SPM or nanomaterials) is expected to display a positive surface charge.

60. Obviously, the introduction of a heteroagglomeration partner into the test system is required. Hence, selecting suitable SPM analogues is the first crucial step. Such analogues need to be stable (for the test duration), reproducible (among test runs) monodisperse and well characterised in terms of composition, size (size distribution), shape and density (to allow a good approximation of the number-based SPM concentration needed for estimation of α_{hetero}, see below). The options may range from very simple mineral analogues (e.g. quartz particles) up to the use of well-characterised natural samples (e.g. river waters or sewage sludge) and the choice of a relevant SPM type depends on the aim of the study.

61. For a general comparative assessment of nanomaterials with regards to their heteroagglomeration behaviour in freshwaters, a simple mineral analogue would be too simplistic, whereas natural water samples do not allow for generalisations. Therefore, in an EU project (NanoFASE) researchers are on the way to create a “model SPM” (complex flock-like SPM analogues that represent process-relevant characteristics of natural SPM) and a standard procedure for its production. These SPM flocks are composed of naturally occurring minerals, selected based on a trade-off between representing the dominant mineral mass fractions and covering a broad range of physicochemical surface properties (e.g. surface charge) in realistic mixing ratios, which are typically encountered in natural freshwater SPM. The mix includes quartz, illite, hematite and organic macromolecules associated with microbial activity. This could be one “standard SPM” to be used in the test, with regards to the requirements for suitable SPM analogues mentioned above.

62. In order to assess the fraction of “free”, not heteroagglomerated nanomaterials after a certain or several (necessary to estimate alpha values) specified interaction time(s), a separation step needs to be introduced. Separation should be fast and non-intrusive, to allow removal of the SPM-attached nanomaterials from suspension while minimising artefacts on the “free” nanomaterial fraction. That can be achieved by centrifugation at each time interval. Gravitational separability of nanomaterials and SPM hence becomes a prerequisite, meaning that the size and/or density of the SPM needs to exceed that of the tested nanomaterials, to an extent at which (at a selected centrifugation speed and time) the SPM will be removed from suspension, while free nanomaterials will not. This can be ensured by employing centrifugation cut-off calculations and verified by preliminary testing. If significant sedimentation of the SPM over the test duration is likely, agitation by shaking or stirring during the reaction time might be necessary. However, it cannot be ruled out that shaking and stirring affects the apparent rates of heteroagglomeration.

63. The selection of the nanomaterial mass concentration needs to be based on the analytical limits in the matrix (as a rule of thumb, the quantification of remaining “free” nanomaterials should be possible down to ~10 % of the initially added nanomaterials). One can either quantify the elemental mass concentration or the particle number concentration in the supernatant by ICP-MS (after digestion) or single particle ICP-MS, respectively.

64. The analytical limits define the minimum initial nanomaterial mass concentration at the start of the experiment. However, homo- and heteroagglomeration kinetics are driven by number concentrations or more precisely the number ratio of nanomaterials and SPM(Labille et al. 2015); as well as the collision rate constant (which depends on the ENP/SPM size/density and the shear rate (G) in the system, see equations 1 in chapter 3.4). Taking the size[2]/density of nanomaterials and SPM as given, the mass-corresponding number concentrations can be calculated assuming spherical shape. Since heteroagglomeration is the process of interest, the selection of the SPM number concentration and shear forces needs to ensure that simultaneously occurring homoagglomeration[3] remains negligible or is at least significantly dominated by heteroagglomeration. Regarding shear forces, which are not allowed in the original TG318, particles in the nm-size range are not affected by shear forces and diffusion is the dominant transport mechanism (Elimelech 1995); meaning that homoagglomeration is independent of G. Hence, optimisation of the test conditions to favour heteroagglomeration over homoagglomeration can be based on model calculations (see equations 1 in chapter 3.4). With the necessary nanomaterial number concentration given (based on analytical limitations), modifying the SPM number concentration and G-value at “worst case” conditions of alpha-homo (attachment efficiency based on homoaggloneration) and α_{hetero} being 1, allows defining suitable conditions. An example is shown in figure 9, where homoagglomeration of 5 ppb 30 nm CeO_2 is certainly dominated by heteroagglomeration when 45 ppm SPM ($d = 6\ \mu m$, $\rho = 1.5 g/cm^3$) is used and stirring which effected an G = 100/s. Additionally, the model gives a first indication of the necessary temporal resolution.

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[2] Note that e.g. light-scattering-based size measurements are biased towards larger sizes and applying such particle diameters may lead to a significant underestimation of the particle number.

[3] Even if homoagglomeration takes place, homoagglomerates may be too small to be removed from suspension by centrifugation.

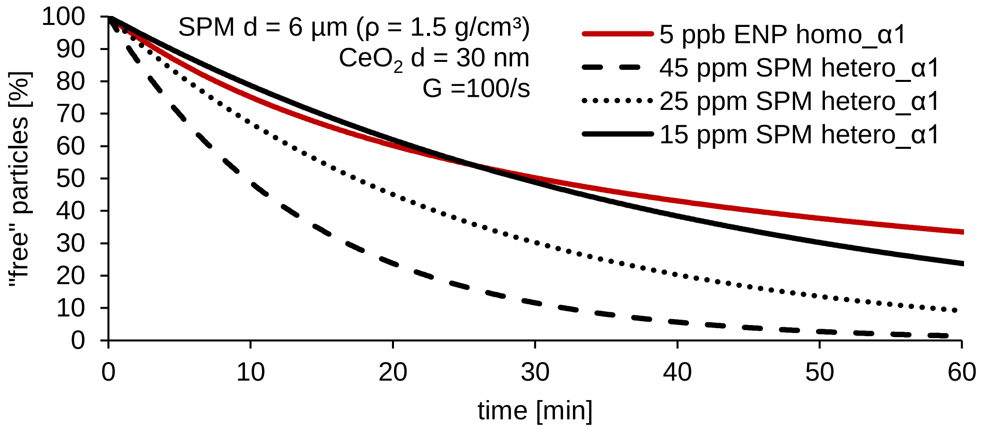

Figure 9. Model calculations to find suitable nanomaterial (ENP) to SPM number concentrations.

65. In order to support the model calculation and the assumption of negligible loss of nanomaterials other than by heteroagglomeration, an additional control test should be conducted. This is done using the same concentration of nanomaterials in the same background hydrochemistry (pH, electrolytes, NOM) but without any SPM. Sampling and sample treatment should be performed in the same way as for the heteroagglomeration test to measure free nanomaterial. Digesting the remaining samples (after centrifugation and sampling supernatant aliquots for free nanomaterial quantification) can serve to close the mass balance and account for losses to the tubes.

66. The test duration window needs to capture the nanomaterial removal over time, which depends on the expected α_{hetero} value, the nanomaterial and SPM number concentrations and collision frequency (equations 1 in chapter 3.4). Hence, the selected particle number concentrations, test-duration and the decision if agitation is necessary or not, can be optimised. . Model calculations may also serve to get an idea about the relevant reaction-time window to be investigated. The more complex and heterogeneous the chosen SPM, the more likely it seems that heteroagglomeration is a very fast process. Thus, it is recommended to aim for a high time-resolution of the initial agglomeration phase (e.g. every few minutes during the first 30 minutes of the test). Intervals can be steadily increased up to e.g. 6 hours and a final "stable" nanomaterial fraction can be determined after e.g. 24 hours.

3.4. Data evaluation and derivation of attachment efficiency

67. Attachment efficiencies, beside the dissolution rate constant, are considered an important parameter for exposure assessment of nanomaterials. During the last years scientists developed fate models for nanomaterials that use attachment efficiencies as input parameters in environmental fate models to predict nanomaterial concentrations in environmental compartments. Such predicted environmental concentrations (PECs) will contribute to nanomaterials risk assessment.

68. Based on Smoluchowski’s agglomeration theory (Elimelech 1995), the heteroagglomeration attachment efficiency (depicted as α_{hetero}) can be defined as the fraction of nanomaterial-SPM collisions resulting in nanomaterial attachment to SPM. α_{hetero} can take values from 0 to 1 to modulate the collision rate constant between nanomaterials and SPM reflecting their physicochemical surface affinity for each other (under the respective hydrochemical conditions). Assuming irreversible attachment, both can be calculated according to equation 1. With a constant number of SPM, heteroagglomeration turns out to be a pseudo first-order reaction (n_{SPM} can be included into k_{het}^{*}) (Praetorius et al. 2012). The collision rate constant (k_{coll}) combines the three modes of transport of nanomaterials and SPM towards each other (diffusion, differential sedimentation and advection), assuming spherical ENP/SPM shape and rectilinear collisions (Praetorius et al. 2012). Transport by advection is incorporated employing the shear rate or the velocity gradient (G) introduced by Camp and Stein (1943) for stirred vessels. It is defined as the square root of the energy (e.g. added by stirring) dissipated by turbulence per unit time, accounting for the volume and viscosity of the considered fluid. The sensitivity of the system to changes in shear is relatively low compared to other parameters[4]. To avoid sedimentation, we suggest a value of G around 40-100 s^{-1} for a stirred batch system. Shear rates in rivers are typically > 10 s^{-1} (Arvidsson et al. 2011).

Equations 1

$$\frac{\mathrm{d}n_{\mathrm{ENP}}}{\mathrm{d}t} = -\,k_{\mathrm{het}}\,n_{\mathrm{ENP}}\,n_{\mathrm{SP}}$$

$$k_{\mathrm{het}} = -\,\alpha_{\mathrm{het}}\,k_{\mathrm{coll}}$$

k_{het}: heteroagglomeration rate constant [s^{-1}]
n_{ENP}: number of nanomaterials
n_{SPM}: number of SPM
α_{het}: attachment efficiency between nanomaterials & SPM
k_{coll}: collision rate constant between nanomaterials & SPM [s^{-1}]

$$k_{\mathrm{coll}} = \frac{2\mathrm{k}T}{3\mu}\,\frac{(r_{\mathrm{SPM}}+r_{\mathrm{ENP}})^2}{r_{\mathrm{SPM}}\,r_{\mathrm{ENP}}} + \frac{4}{3}G(r_{\mathrm{SPM}}+r_{\mathrm{ENP}})^3 + \pi(r_{\mathrm{SPM}}+r_{\mathrm{ENP}})^2|v_{\mathrm{SPM}}$$

$$v_{\mathrm{SPM;\,ENP}} = \frac{2}{9}\frac{\rho_{\mathrm{SPM;\,ENP}}-\rho_{\mathrm{L}}}{\mu}\,\mathrm{g}\,r^2_{\mathrm{SPM;\,ENP}}$$

$$\overline{G} = \sqrt{\frac{\epsilon}{\nu}} = \sqrt{\frac{P}{\mu V}}$$

k: Boltzmann’s constant [m²kg/s²K]
T: absolute temperature [K]
μ: dynamic viscosity of dispersion medium [Ns/m²]
r: particle/aggregate radius [m]
G: shear rate (velocity gradient) [s^{-1}]
g: gravity acceleration [m/s²]
ρ_S: density of particles [kg/m³]

ρ_L: density of liquid [kg/m³]
$u_{SPM;\,ENP}$: settling velocity of SPM or nanomaterials [m/s]
ϵ: power input per unit mass [Nm/skg]
v: kinematic viscosity (μ/ρ_L) [m²/s]
P: power dissipated by liquid turbulence [W]
V: volume of liquid [m³]

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[4] For the model calculation above, a quite wide range of G (shear rate) from 50/s to 150/s would change alpha-hetero by a factor of max. ~2.4 (e.g. from alpha 1 to 0.42).

69. These equations may on the one hand be used to design the tests (SPM and nanomaterial number concentrations, test duration and time resolution, see above) and on the other hand to approximate α_{hetero}. With heteroagglomeration being a pseudo first-order reaction (in the early aggregation phase), the natural logarithm of the particle concentration plotted over time will give a linear relation (see example in Figure 10). The slope equals the pseudo first-order reaction rate ($k_{het}{}^{*} = -\alpha_{het}\, n_{SPM}\, k_{coll}$) and α_{hetero} can be calculated.

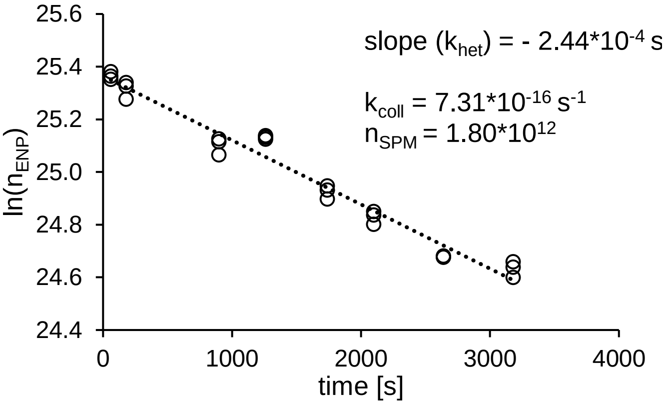

Figure 10. Pseudo first-order kinetics of heteroagglomeration

70. As there will be many collisions between nanomaterials and SPM, only nanomaterials with very low attachment efficiencies are likely to remain “free”. Many results (e.g. Praetorius et al. 2012) have shown alphas that result in almost immediate nanomaterial-SPM associations. The exception is, if the SPM number concentration is very low. In the latter case homoagglomeration will be dominant (see above). Assuming pseudo first-order kinetics, the half-life of free nanomaterials can be calculated ($t_{1/2} = \ln(2)\,/k_{het}{}^{*}$). Plotting the expected half-lives at given SPM/nanomaterial concentrations and shear rate, over a range of alpha-values employing three different SPM concentrations (Figure 11) shows that only starting from small alpha <0.01-0.001 significant numbers of free nanomaterials will remain after just one day, except for very low SPM concentrations (5 ppm and lower).

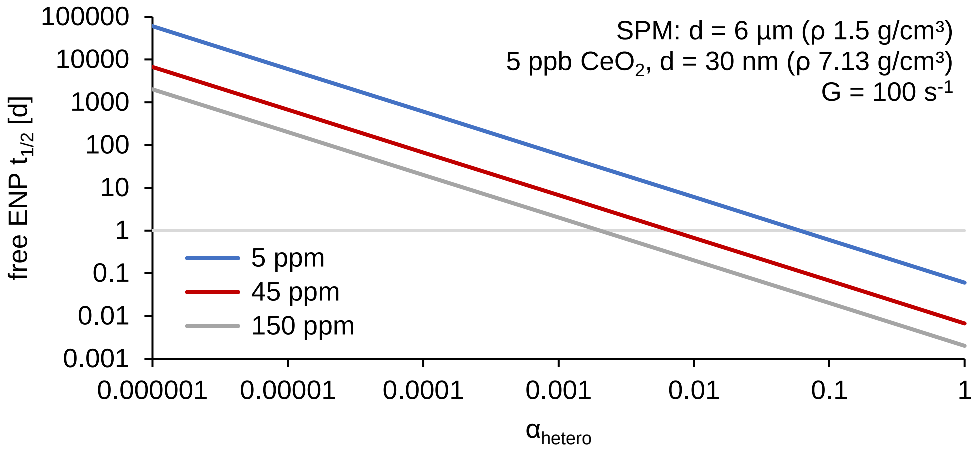

Figure 11. Relation of the pseudo first-order half-life of free nanomaterials (irreversible attachment) and α_{hetero}. The number of SPM (n_{SPM}) and k_{coll} were calculated with the given parameters assuming spherical shape.

71. Based on the considerations above, the test duration of 6 hours defined in TG 318 will likely also work for heteroagglomeration. For conditions where heteroagglomeration is the dominant process, transport-relevant α_{hetero} values of 0.01 and lower are only to be expected if there is a significant fraction e.g. 10-20 % of nanomaterials remaining after 3 (conservative) to 6 hours. With further experimental data providing proof of principle, this relationship between the order of magnitude of α_{hetero} and the test duration can be further refined.

72. A suggestion for a first tier assessment could be a two-point measurement at t_0 and e.g. t = 2 h, to assess if there is a fraction of >10% of the nanomaterials left (as compared to t_0[5]). If that is the case, there is a chance that α_{hetero} is < 0.01-0.001 and a kinetic study should be conducted. Based on such an approach a comparison of different nanomaterials tested under the same conditions, analogously to the homoagglomeration test (TG 318), can be established e.g. less than 10% left after 2 h (= fast heteroagglomeration: alpha >0.1 and $t_{1/2}$ < 1d expected), more than 10% left after 2 h (intermediate heteroagglomeration: kinetic study and alpha determination). A next useful step could be a determination of a "final" stable fraction (e.g. after 24 h). Studies found that removal of nanomaterials can reach a plateau (Quik et al. 2012 and 2014), which is likely a sub-fraction of the added nanomaterials that either had different intrinsic surface

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[5] A comparison to t_0 might be substituted by a comparison to a "homoagglomeration" control (containing the same amount of nanomaterials with the same background hydrochemistry and DOM concentration, but without SPM). However, using the t_0-heteroagglomeration concentration as reference for comparison ensures that, at both time points, the same fraction of free nanomaterials was removed by screening-effects (SPM physically screening and thus removing nanomaterials during centrifugation). This helps to avoid a misinterpretation of a screening-induced removal of nanomaterials from suspension as a heteroaggregation-induced removal. However, if heteroagglomeration is very fast, a homoagglomeration control might provide a more reliable initial concentration (but only if the screening effect can be considered negligible, e.g. when very low SPM concentrations are used).

properties, or was permanently stabilized by the testing-matrix (e.g. NOM). This would be the very stable fraction that is unlikely to undergo heteroagglomeration on the long run and shall to be considered “free” nanomaterials when it comes to transport regimes.

4 Use of data generated on dissolution testing and dispersion stability for further testing and assessment strategies

73. Both dispersion stability and dissolution (rate) are considered to be endpoints of major importance for describing environmental behaviour and fate of nanomaterials. Thus, for fate assessment as well as for exposure assessment both endpoints need to be taken into account, and they will also be key information for ecotoxicity testing.

74. While TG 318 for dispersion stability has been published (OECD, 2017), currently no OECD TG on solubility and dissolution rate of nanomaterials is available. However, existing guidance documents like the GD 29 (OECD 2001) may provide a basic orientation (see Chapter 2). Other TG and GDs as well as ongoing WNT projects are also dealing with areas of dissolution (see Chapter 2 and Chapter 6).

75. There is not only one single optimal strategy on how to determine solubility, dissolution, and dispersion stability. The starting nanomaterial concentrations, the media composition, and timeframe that is relevant to use will always depend on the purpose and objective of testing (see Section 4.1). Likewise, the testing strategy employed depends on whether the data is needed to inform consideration of what further ecotoxicity testing is needed, as input data for modelling of environmental fate, or as basic data on environmental behaviour (see Section 4.2).

76. Validity and correct interpretation of dispersion stability, and dissolution rate or solubility measurements are interdependent. For instance, the measurement of dissolution in an unstable suspension where agglomeration and sedimentation has occurred will underestimate dissolution (as agglomerated/precipitated material will have less surface available for dissolution). Measurement of dispersion stability based on remaining particulate fraction where dissolution has occurred will underestimate dispersion stability as any nanomaterial missing through dissolution will be interpreted as precipitated. Further situations where such caution must be shown when interpreting the data and where both types of data must be viewed in concert are highlighted with some outline application examples in Section 4.3.

4.1. Purpose

77. Dissolution (rate) and dispersion stability are relevant triggers needed for the safety assessment of nanomaterials. Regulatory information requirements for solubility, dissolution rate and dispersion stability of nanomaterials exist in some chemical legislation such as REACH (EU 2018). These parameters are also relevant as triggers for further assessment strategies and decisions. Giving the current worldwide development in chemicals regulations towards specific provisions for nanomaterials (e.g. US EPA, 2016), it is anticipated that other chemicals legislations meet the same challenges.

78. The understanding of environmental fate and behaviour of nanomaterials is one of the key aspects for environmental exposure assessment. Furthermore, it supports the knowledge about uptake by, interaction with, and effects on environmental organisms. Therefore, dissolution, (hetero-) agglomeration and sedimentation are important drivers for environmental fate, behaviour and effects of nanomaterials.

79. Furthermore, given the numerous manufactured nanomaterials already on the market and many more expected in future, the effort for the individual investigation of hazard and risk would be enormous and virtually impossible. To overcome this challenge, grouping- and analogy approaches of nanomaterials has been identified as one critical issue by OECD (OECD 2016). Grouping and read-across approaches are already established for chemical substances to meet regulatory data requirements by deviation from the requirements of performing standard test for years (OECD 2007, 2014c). The aim of these approaches is to predict the physico-chemical, toxicological, and fate-related properties of chemical substances based on structural similarities. If sufficient evidence for similarity is available between two chemical forms, then it should be possible to transfer available data on the fate or hazard of one chemical substance to another one. These approaches are intended to reduce the amount of testing necessary to determine the behaviour and effects of all individual members of a group.

80. Grouping and read across based on molecular structural similarity alone is not sufficient for nanomaterials, as nanomaterials of the same chemical composition but deviating in physical-chemical parameters can show differences in effects and behaviour. To allow an adequate assessment of nanomaterials using grouping and read-across further parameters are necessary to justify such an approach. In that context, beside chemical composition, parameters like morphology, surface properties, and shape as well as reactivity, and fate descriptors like dissolution rate or dispersion stability in relevant media are discussed for consideration (e.g. ECHA Guidance R6.1 (ECHA 2017)).

81. TG 318 for dispersion stability and the available methods for solubility and dissolution rate can address these very endpoints and might be used to compare similarity or differences of nanomaterials regarding these endpoints at given conditions and thus support the development of grouping and analogue approaches for nanomaterials as part of integrated testing and assessment approaches (IATAs).

82. For further (hazard) testing knowledge on dispersion stability and dissolution behaviour can inform on the test set-up and necessary measurements for exposure characterisation during the testing. Furthermore, knowledge on dispersion stability and dissolution behaviour in a test system can facilitate better interpretation of test results (e.g. whether effects seen can be attributed to nanomaterials or free ions only).

4.2. Testing strategy

83. Based on the purpose of testing information from solubility, dissolution rate and dispersion stability testing can inform a further testing strategy. The suggested approach to do so is outlined in Figure 12. The approach starts with a screening batch test on dissolution followed by a dynamic dissolution test if needed. The approach furthermore includes the testing of dispersion stability for nanomaterials that do not fully dissolve within a relevant time scale. This approach also provides further information to guide on the effort needed for appropriate analytics in further testing.

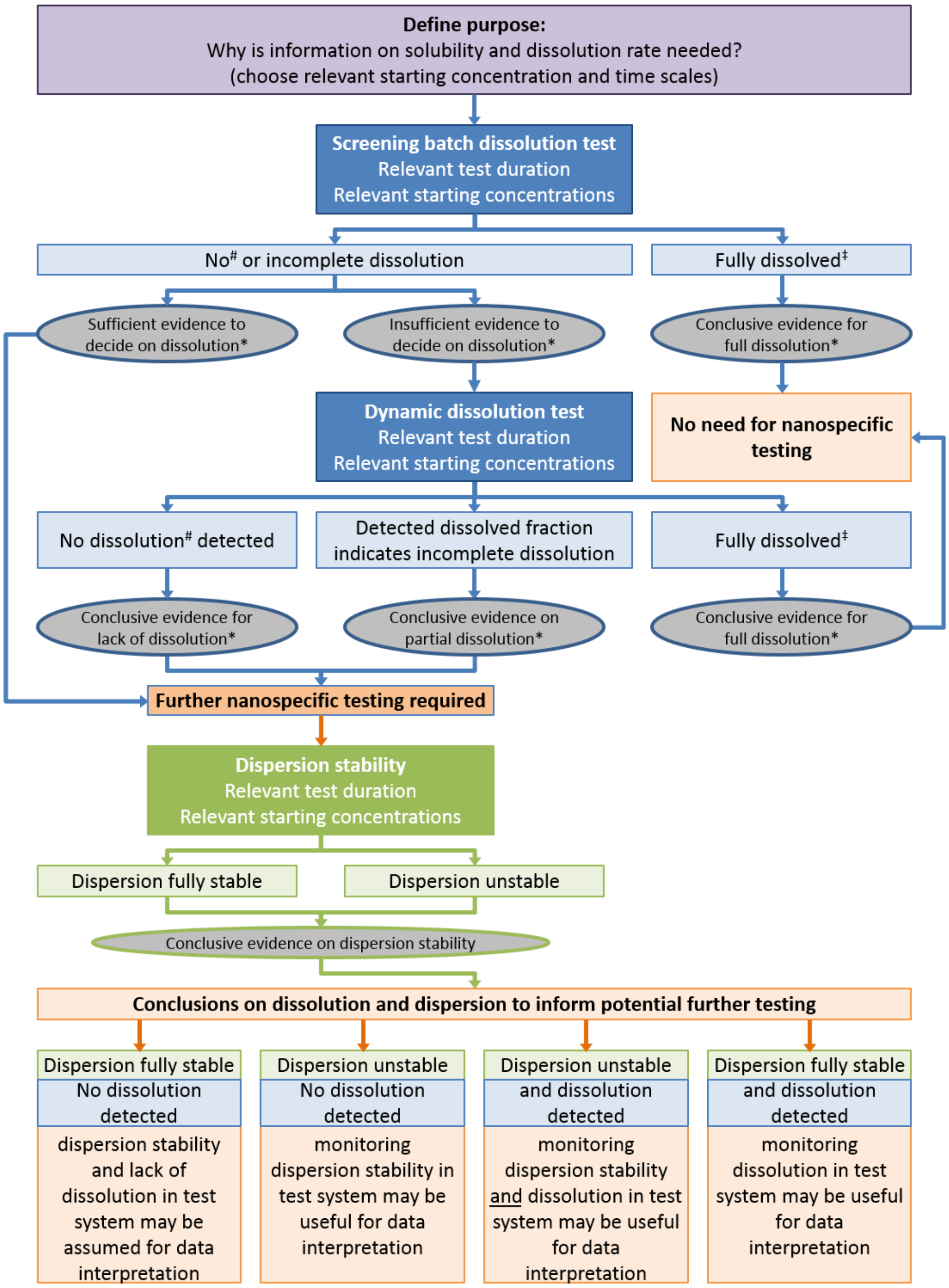

Figure 12. Testing strategy building on dissolution and dispersion stability data. (Legend: # below detection limit; ‡ all relevant mass in dissolved fraction; * in relevant time scale)

84. Depending on the nanomaterial under investigation and the objective of the testing, appropriate starting concentrations and test duration need to be chosen for the initial screening dissolution batch test. When addressing triggers for safety assessment and decision making for further testing, there might be the need to test concentrations which are beyond environmental realism. Therefore, it is advisable to test the nanomaterials with

test conditions of TG 318 and the upcoming TG for dissolution, respectively, to gain comparable and reliable data. Furthermore, it might be challenging to analytically verify test concentrations under (mimicked) realistic environmental conditions.

85. In the case in which the batch test results in conclusive evidence for full dissolution (all relevant mass in dissolved fraction) of the investigated nanomaterial in a relevant time scale, further dissolution testing by dynamic methods might not be needed. In this case also refrainment from nanospecific testing can be considered. For nanomaterials that show incomplete dissolution or dissolution below detection limit, it should be decided if the information from the batch test is sufficiently evident to decide on dissolution in respect of the purpose of testing. For example, information on dissolution based on the batch test could provide sufficient supplementary information for a subsequent ecotoxicity test. In contrast, information from batch test might be insufficient to e.g. inform exposure relevant testing regimes in which dissolution against an infinitive sink is of interest.

86. In case information from the batch test is sufficient to conclude on the dissolution of the investigated nanomaterial one could directly proceed with further nanospecific testing. If the information from the batch test is insufficient, testing of dissolution with a dynamic method should be considered. For nanomaterials that do not fully dissolve by a dynamic dissolution test within a relevant time scale, again further nanospecific testing is needed. For further nanospecific testing a first suggested step is testing of dispersion stability.

87. For testing dispersion stability, it is recommended to use OECD TG 318 as it is described. Deviation from test conditions might be possible in order to inform higher tier testing. In the latter case it is suggested to choose appropriate test duration and concentration based on the objective. Information from dispersion stability testing can provide an indication about the analytic needs (monitoring of dissolved fraction, monitoring of dispersion stability) to be performed for or during potential further nanospecific testing.

88. In addition, results from solubility, dissolution rate and dispersion stability testing can provide information to facilitate the choice of appropriate test performance of potential further testing (e.g. frequency of water renewal rate, (semi)static vs. flow through test conditions) to maintain stable conditions.

4.3. Points to consider when applying information on dispersion stability and dissolution for potential further testing

89. The methods presented in this guidance document will provide relevant data on solubility, dissolution and dispersion stability. Moreover, in principle these methods can also be performed with test media (e.g. natural water, ecotoxicological test media) differently compared to the ones presented in the respective test guideline or recommended here. These endpoints are influenced by extrinsic and intrinsic properties of the nanomaterial in question and therewith results can differ depending on nanomaterial modification and the test media used.

90. Hence, at the same time the repeatability, reliability, and robustness should be taken into account as much as possible. When using alternative test conditions, it is prerequisite to characterise and report the test media conditions to enable data evaluation

and interpretation of data (and consider simultaneous testing). This will also enable retrospective analysis of studies compared with new data produced in the future.

91. While information from these methods might be directly applicable for exposure estimation, conclusions for a further testing strategy on hazard should be considered carefully. The relevance of the information as well as the decision how to consider data generated on these endpoints for further testing will always depend on the individual question and purpose of testing.

92. Solubility and dissolution rate inform about in which state (particulate, completely or partly dissolved) a nanomaterial is present under respective conditions. Regarding solubility and dissolution rate the main question is when it is possible to refrain from nanospecific considerations in hazard assessment (i.e. when dissolution is considered complete and fast enough). For an appropriate decision on the relevant hazard assessment, various aspects need to be considered like the regarded nanomaterial (e.g. chemical composition), endpoint under investigation (e.g. long term vs. acute testing), and test performance (e.g. media type, water renewal frequency). In sense of an aquatic hazard endpoint it might be possible to refrain from nanospecific testing, if the nanomaterial under investigation fully dissolves at the highest corresponding test concentration of the respective ecotoxicity test within 24 h. For such cases dissolution screening testing in the relevant media is needed to investigate dissolution within 24 h. The rationale for this timeframe of investigating dissolution is based on test durations of many short term tests and suggested water renewal frequencies in most (semi-)static tests which usually cover 24 h. In such cases, the contribution of the particulate form to the eco-toxicological impact will very likely be not decisive.

93. OECD TG 318 provides information on how stable a nanomaterial dispersion is in aqueous media at given conditions. There is sufficient evidence that nanomaterials tend to agglomerate and settle out of the water phase during sufficient long periods and thus sediment, sludge and soil are expected to be the main environmental target components (Hartmann et al. 2014). Thus, if nanomaterials show low stability in the water phase, inclusion of data relating to the sediment or soil compartment into environmental risk assessment of nanomaterials may become relevant.

94. However, agglomeration and sedimentation should not be equated per se with lack of exposure in water column. For hazard testing, existing data show that agglomeration and sedimentation do not necessarily prevent availability to the organisms in aquatic test systems. Due to the permanently agitated test system used for testing algae toxicity, test organisms also can be affected by nanomaterials unstable in the test dispersion In addition, unstable dispersed nanomaterials can be taken up by pelagic organisms like daphnia and fish (Botha et al. 2016, Tan et al. 2016, Hund-Rinke et al. 2018, Johnston et al. 2010). Nanomaterials furthermore can be available to deposit-feeders and filtering organisms (Marie et al. 2014, Kühr et al. 2019). In case of ion releasing nanomaterials, released ions might get back into the water phase, affecting test organisms while the particulate fraction remains deposited at the bottom. Strictly speaking this may not be considered nanomaterial toxicity, but by constantly replenishing the ion concentration in the water phase, the particulate fraction strongly influences the outcomes of the test. Also under environmental conditions it is conceivable, that deposited or heteroagglomerated nanomaterials are consumed from biofilms, ground or plant surfaces or taken up via sediment or natural suspended matter, respectively (Geitner et al. 2018, Perrier et al. 2018). The understanding of bioavailability and uptake of the various nanomaterials by aquatic organisms is also still far from complete and – besides dissolution and agglomeration – also depends on other

aspects of the nanomaterials like size, chemical composition, synthesis methods, or nature of coating of the investigated nanomaterial (Lead et al. 2018).

95. When using data from dispersion stability and dissolution testing for exposure assessment it has to be taken into account that not only a singular but also a continuous entry into the environment is of relevance. This can lead to a situation that even if a nanomaterial dissolves relatively fast a mixed exposure of particulate and dissolved forms can take place.

96. For nanomaterials that release ions or dissolve over a sufficient long period of time, it is important to monitor in-depth the dissolution in the test system to inform on the amounts of particulate and dissolved forms being present. It might be also of importance to know how the particulate form changed in composition or size distribution. Changes in composition might be relevant for e.g. nanomaterials that consists of various substances (e.g. core-shell nanomaterials) with different dissolution behaviour or nanomaterials with surface modification. Knowledge on changes in size distribution would provide details on how size distribution changes (e.g. loss of smallest particle, decrease of particle size across the distribution, formation of new particles) and might be relevant to decide on further hazard and fate testing strategies.

97. The joint consideration of both endpoints determined by the TG 318 and available methods on dissolution is of expedient importance as both endpoints strongly influence each other and interpreting one in absence of the other is difficult. The dispersion state of a nanomaterial will affect its dissolution, while dissolution of the nanomaterial will alter the outcome of dispersion stability testing for dissolving nanomaterials.

98. Dissolution leads to an increase of ion concentration that in consequence can lead to either an increased or a decreased dispersion stability. Furthermore, readily dissolution can lead to a fade of particles during TG 318 performance. Therefore, care should be taken when analysing and interpreting of results from TG 318 to not mistake instable dispersion with dissolved nanomaterials. In TG 318 it is described that dissolution needs to be evaluated during testing for ion releasing nanomaterials. On the other hand, agglomeration/sedimentation will lead to a decrease in available surface, which in consequence will result in a decrease in dissolution.

99. Next to (hetero-)agglomeration, sedimentation and dissolution, also potential nanomaterial transformations (e.g. sulphidation, oxidation/reduction reactions and coating transformations) influence the fate of nanomaterials in the environment and thus should be considered when evaluating the data. Currently, in a foreseen WNT project 3.16 a GD on transformation of nanomaterials in aquatic environmental media will be developed to provide advice on ways to determine abiotic transformations of nanomaterials in the environment.

5 Use of data on dispersion stability, solubility and dissolution rate in exposure modelling

100. Both data from TG 318 and the currently available methods for determining solubility and dissolution rate may support the derivation of appropriate input parameters for exposure modelling of nanomaterials. As a starting point for environmental fate modelling, basic data as collected using TG 318 and adapted test performance of GD 29 might be used as input for deriving screening level estimations of predicted environmental concentrations (PECs), e.g. to compare potential exposure of various nanomaterials. For refinement of such data, further testing like using dynamic test designs to determine dissolution (rate) and/or alternative media (e.g. in order to determine heteroagglomeration with TG 318) will help to improve the PEC estimations in various environmental compartments.

5.1. Use of data on dissolution rate in exposure modelling

101. Data on dissolution of nanomaterials is essential in environmental exposure to enable distinction between the dissolved and the particulate form of a chemical or nanomaterial. This distinction is also made in current environmental fate models for nanomaterials, where dissolution is a process by which nanomaterials are transformed from the particulate form to the dissolved form (Meesters et al. 2014, 2019). As discussed in Chapter ~~3~~ 2 dissolution data can be used to estimate the dissolution rate constant. This is an input parameter in environmental fate models to calculate the predicted exposure concentration. Assuming first order kinetics, the dissolution rate constant can be calculated from experimental data as:

$$k_{\mathrm{diss}} = \frac{ln\left(\frac{[\mathrm{ENP}_0]-\left([\mathrm{M}^{n+}]-[\mathrm{M}_0{}^{n+}]\right)}{[\mathrm{ENP}_0]}\right)}{t}$$

where $[\mathrm{ENP}_0]$ is the nanomaterial concentration in mg/L at the start of the experiment ($t = 0$), $[\mathrm{M}^{n+}]$ is the ionic metal concentration in mg/L at time t, $[\mathrm{M}_0{}^{n+}]$ is the ionic metal concentration in mg/L at the start of the experiment ($t = 0$), (k_{diss}) is the dissolution rate constant, and t is time of measurement.

102. As k_{diss} is only derived from ions appearing in solution over time, it is essential that the measurement technique used enables a distinction of the ionic fraction from the other metal fractions. It should be considered to use the ion concentration in the untreated medium as $[\mathrm{M}_0{}^{n+}]$ to ensure that this value is not influenced by ENP_0. Some caution is needed where rapid dissolution occurs at the beginning of the experiment, as this may result in artificially low k_{diss} when measurements do not capture this (e.g. when measurements are only available for 0, 24, and 48 hours).

6 Links to other relevant TGs and GDs

6.1. WNT project 2.51: GD on Aquatic and Sediment Toxicological Testing of Nanomaterials

103. Information on nanomaterial dissolution and dispersion stability should be used in addition to the guidance given in the GD on aquatic (and sediment) toxicity testing of nanomaterials (currently under development) in order to interpret the determined aquatic and sediment toxicity data. In the present GD (Chapter 4) a testing strategy is suggested for solubility, dissolution rate, and dispersion stability to inform about in which state (particulate, completely or partly dissolved) a nanomaterial is present under conditions of an ecotoxicity study and help to interpret test results regarding the contribution of dissolved or undissolved fraction to the toxicity.

104. Information on dispersion stability helps to identify the main routes of exposure within the test system (e.g. via water phase in case of stable dispersion, via possible dietary exposure of unstable dispersion, via sediment).

105. In addition, results from solubility, dissolution rate and dispersion stability testing can provide information to facilitate to decide on the analytic needs and the choice of appropriate test performance of potential further testing (e.g. frequency of water renewal rate, (semi)static vs. flow through test conditions) to maintain stable conditions.

106. Data on heteroagglomeration obtained using this GD may also support interpretation of ecotoxicological data of standard test results. SPM of different forms are present in high enough concentrations in some standard tests and can affect the nanomaterial that organisms are exposed to. Attachment to fish feed, if it occurs, determines how fish are exposed to nanomaterials, via gills or via the gut (Handy *et al.*, 2018). Extracellular polymers e.g. algae exudates may occur in standard aquatic tests and heteroagglomeration of nanomaterials and such polymers affects the exposure and consequent toxicity of nanomaterials to the organisms (Yang et al., 2018).

107. Even though tests in soil systems are not included in the GD on aquatic and sediment tests, the recommendations mentioned above also apply to soil organisms. Soil pore water is expected to be an important exposure pathway of nanomaterials to soil organisms. Heteroagglomeration and dissolution in soil pore water is therefore also determining the toxicity to soil organisms. Attachment to inorganic particles such as clays or iron oxides is likely in standard soils (Miglietta *et al.*, 2015) and determines whether organisms are exposed to individual nanomaterials and/or to heteroagglomerated nanomaterials, which may affect toxicity (Gupta et al., 2017).

108. To broaden the significance of data on dispersion stability or dissolution for interpretation of nanomaterial behaviour in ecotoxicity test media, the simulated environmental media used in TG 318 and for dissolution testing may be amended to determine the dispersion and dissolution behaviour directly in the relevant test media for ecotoxicity testing.

109. Comparing information on dissolution (rate) and dispersion (stability) of different nanoforms of a substance prior to ecotoxicity testing of those nanoforms, can support the development of hypotheses on expected similar behaviour and thus may guide grouping and read across.

6.2. WNT project 3.14: GD to support the use of TG312 (-Leaching in soil columns) for nanomaterial safety testing.

110. A GD on how to conduct column tests to determine transport of nanomaterials in porous media based on TG 312 (OECD, 2004) was being developed at the time of drafting and includes recommendations on how to calculate a global attachment efficiency (α_{global}) for a specific nanomaterial in a specific soil. This GD provides guidance on calculating the attachment efficiency for interaction between a specific nanomaterial and specific SPM (α_{SPM}).

111. It has to be noted that these attachment efficiencies are not the same, even if SPM material is used that is relevant for the specific soil studied. α_{global} is a lumped parameter grouping many different processes that reduce transport (and thus increase α_{global}) in soils (Baalousha et al., 2016). One of these processes may be heteroagglomeration with native SPM in the pore water, the rate quantification of which may occur as described in this GD.

6.3. WNT project 3.12: Assessing the Apparent Accumulation Potential of Nanomaterials during fish bioaccumulation studies

112. As described earlier, dispersion stability and ion dissolution will affect what the organisms are exposed to, both in standard tests as in realistic environments. Interpretation of bioaccumulation testing therefore likewise needs information on dispersion stability, heteroagglomeration and dissolution of the nanomaterial in question as, for instance, proposed for fish bioaccumulation testing (Handy et al. 2018).

113. A dissolution rate rather than solubility is needed for bioaccumulation testing given the kinetic nature of bioaccumulation.

114. The information from dissolution, dispersion stability, and heteroagglomeration could also be used for decision making prior to testing, or bioaccumulation test media could be used in an adapted protocol of both endpoints in order to investigate dispersion and/or dissolution under experimental settings relevant for bioaccumulation tests.

6.4. OECD TG 105: Dissolution in water

115. TG 105 (OECD, 1995) describes a column elution method and a flask method to investigate solubility of compounds.

116. During the column elution method of TG 105, the solubility of a solid chemical is determined while attached to an inert substrate in a column. The solubility equals the plateau concentration of a chemical dissolved from the test material reached in the eluate of the column.

117. The flow-through testing for dissolution rate described in this GD is similar to the column elution method of TG 105 in that sense that the simulated media in contact with the nanomaterials is continuously refreshed and the concentration of dissolved substances is measured continuously. However, it is not clear how nanomaterials could be attached to such a substrate. In this GD, the nanomaterials are therefore attached to an ultrafiltration membrane or contained in a compartment formed by ultrafiltration membranes that are continuously in contact with the medium.

118. The data interpretation in this GD is fundamentally different from that in TG 105. TG 105 describes how the solubility of compounds can be obtained, whereas this GD describes how a dissolution rate can be obtained.

119. The flask method in TG 105 resembles the static batch test method described in this GD. However, TG 105 prescribes that solution and solid material must be separated using centrifugation after 24 hours of equilibration after which the solubility is measured. This GD prescribes using ultrafiltration as a separation technique, because centrifugation does not guarantee separation of nanomaterials and dissolved substances. Moreover, this GD encourages dissolved concentrations to be measured at several time points allowing to fit a kinetic model and calculating the dissolution rate.

6.5. OECD GD 29: Guidance document on transformation/dissolution of metals and metal

120. Similarly to ~~the~~ this GD, GD 29 (OECD, 2001) describes a screening level and one full dissolution/transformation test. A high loading of a solid compound is equilibrated for 24 hours in a test medium during the screening test followed by a single measurement.

121. The dissolved fraction is determined in a 0.20 mm filtrate using GD 29, differing from this GD where ultrafiltration is used for separation, because nanomaterials may pass a 0.20 mm filter. Further, indications on adapted needs when considering nanomaterial testing can be found in chapter 2.

122. In the full test described by GD 29, three different concentrations of a solid compound are equilibrated at times up to 28 days, where the test medium is refreshed every 24 hours. Refreshing the medium would not be possible for nanomaterials in this way, because they are suspended in the medium and would be removed during refreshing.

123. This GD recommends continuous refreshing, more in line with TG 105 and ISO TR 19057 (ISO 2017). As in the screening test, ultrafiltration is recommended in this GD.

6.6. OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method

124. TG 106 (OECD, 2000) describes a batch method where a substrate, usually a soil powder, is equilibrated with a chemical dissolved in water for a determined time. The chemical then adsorbs and this process is quantified in the form of a partitioning coefficient (K_d). This GD does not pertain to dissolved chemicals but to nanomaterials that will not establish an equilibrium with any substrate (Praetorius et al., 2014). An attachment efficiency is deducted in this GD using a kinetic approach instead of a partitioning coefficient.

125. Finally, SPM is recommended as a substrate in this GD, whereas a soil is usually used for TG 106.

6.7. OECD WNT project 3.11. TG for nanomaterial removal in wastewater

126. This TG development project aims to provide a standard method for characterising the interaction between nanomaterials and wastewater treatment sludge. This process is thus similar to heteroagglomeration tested in this GD, but the SPM in the case of WNT project 3.11 (OECD, 2018) is specific biomass sampled from a wastewater treatment plant.

127. The approach in WNT project 3.11 prescribes mixing biomass with nanomaterials for one hour followed by gravitational settling. The test is done at different total nanomaterial concentrations and sorption isotherms are calculated based on the nanomaterial concentration measured in the supernatant after settling. This GD conversely recommends separating free from heteroagglomerated nanomaterials by means of accelerated sedimentation using centrifugation.

128. The parameter reported from this GD in terms of heteroagglomeration is attachment efficiency which is a dimensionless parameter that is used in kinetic models, i.e. models that assume equilibrium does not last. In contrast sorption isotherm reports isothermal trends of partitioning coefficients and the underlying model of these parameters assume an continuously equilibrium or steady-state situation. Different parameter are thus determined.

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Annex I Terminology, definitions and abbreviations

129. One important issue is the **harmonization of terminology and definitions** in various TGs and GDs for environmental fate testing of nanomaterials. Currently the published OECD TG 318 (OECD 2017) for dispersion stability already contains agreed and adopted terminology and this should be currently followed in future TGs and GDs when applicable.

Agglomeration – Process of contact and adhesion whereby dispersed particles are held together by weak physical interactions ultimately leading to enhanced sedimentation by the formation of precipitates of larger than colloidal size (agglomerates) (slightly modified according to 7). In contrast to aggregation where particles held by strong bonds like sinter bridges, agglomeration is a reversible process.

Alpha value – nanomaterial affinity efficiency in various conditions (homoagglomeration and heteroagglomeration)

BET – Gas-adsorption method to determine the specific surface area of powders according to the Brunauer–Emmett–Teller theory.

CCC – critical coagulation concentration: the concentration of a specific counter-ion (cation for negatively charged particles, anion for positively charged particles) at which the repulsive forces between particles approach zero and the agglomeration enters a fast, diffusion limited regime. At the CCC the attachment efficiency α becomes 1 and the stability ratio W zero.

Concentrations – Concentrations of the particles and NOM in the samples are given in grams per litre (g/L), milligrams per litre (mg/L) and micrograms per litre (µg/L). In case of NOM it is given in mg/L of DOC. Concentrations of electrolytes ($Ca(NO_3)_2$ and $NaHCO_3$) present in the samples are given in millimole per litre (mM/L).

DOC – Dissolved Organic Carbon: the organic bound carbon in a water sample which passes a 0.45 µm filter, a part of the DOM. DOM in the form of humic substances have ~50 % DOC.

Dispersibility – is the condition of particular material of being dispersible or a measure to which extent it is dispersible in a dispersing medium or continuous phase. Dispersion stability refers to the ability of a dispersion to resist change in its properties over time.

Dissolution – Dissolution is understood as a process under which a substance solves.

Dissolution rate - is dissolution over time depending on e.g. available surface area of the material.

Heteroagglomeration – Agglomeration of particles (here nanomaterials) with other particles (synthetic or natural).

Homoagglomeration – Agglomeration of particles (here nanomaterials) with each other

IEP – Isoelectric point: the pH at which the zeta potential or particle mobility in response to an electric field is zero. Only in the absence of specifically adsorbing ions which change the surface charge compared to the pristine surface, the IEP equals the PZC.

NOM – Natural organic matter: the organic substances present in surface or ground water. NOM covers humic and non-humic fractions as i.e. polysaccharides. NOM is operationally divided into Dissolved Organic Matter (DOM) and Particulate Organic Matter (POM). DOM passes a 0.45 µm filter, POM is retained by the same filter. POM as defined herein should not be confused with purgeable organic carbon.

PZC – Point of Zero Charge: the point where the surface charge density is zero in the absence of any specifically adsorbing ions. Its value is given as the negative logarithm of the potential-determining ion. In most cases the potential determining ion is the proton and the value is then given as pH. The PZC is then also termed the PZNPC – point of zero net proton charge.

Size – Size of the particles, aggregates or agglomerates is given in micrometres (µm) or nanometres (nm). The method for particle size determination and the character of the particle size average should be reported.

Solubility - Solubility is an ability of a substance to dissolve in a solvent (max amount that can be solved under certain conditions) and this depends on chemical composition.

SPM – Suspended particulate matter

SRNOM – Suwannee River NOM: a standard surface water NOM material of the International Humic Substance Society (IHSS), isolated from the Suwannee River (US) by reverse osmosis and purified according to the procedures of the IHSS. The material can be purchased from the IHSS.