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Total number of authors:  
12

Published in:  
Environmental Science: Nano

Link to article, DOI:  
[10.1039/D0EN01123A](https://doi.org/10.1039/D0EN01123A)

Publication date:  
2021

Document Version  
Peer reviewed version

[Link back to DTU Orbit](#)

### Citation (APA):

Nielsen, M. B., Baun, A., Mackevica, A., Thit, A., Odnevall Wallinder, I., Gallego, J. A., Clausen, L. P. W., Rissler, J., Skjolding, L. M., Castro Nilsson, A., Cedervall, T., & Hansen, S. F. (2021). Nanomaterials in the European chemicals legislation – methodological challenges for registration and environmental safety assessment. *Environmental Science: Nano*, 8, 731-747. <https://doi.org/10.1039/D0EN01123A>

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# Nanomaterials in the European chemicals legislation – methodological challenges for registration and environmental safety assessment

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## Abstract

In the European Union the Annexes of its chemical legislation (REACH) were revised and now clarifies the technical data requirements for nanomaterials (NMs). These new provisions, effective from January 1, 2020, introduce requirements for manufacturers, importers and downstream users regarding registration and safety assessment of NMs. This study aims to assess the availability and suitability of methods needed to comply with the new regulatory provisions on NMs for physico-chemical characterisation and environmental fate and effects. The scientific literature and relevant test guideline frameworks were reviewed to identify applicable methods. These were subsequently evaluated and categorised as either: ‘internationally accepted test guideline or standard (TGS)’, ‘internationally accepted test guideline or standard under development (TGSUD)’, ‘established as standard methods in scientific literature (SCI)’, ‘other methods and/or more research needed (O)’ or ‘no method (N)’. We find that 80% of the information requirements and a bit more than 40% of the waiving criteria in the new REACH Annexes are supported by methods that are available as TGS, TGSUD or SCI. Most of the relevant methods in the scientific literature are included in recent OECD guidance documents or ECHA guidance. We recommend that a targeted effort is made to develop protocols and guidelines for methods to determine NM adsorption/desorption, degradation, exposure scenarios and ability to cross biological membranes. Here methods to fulfil the information requirements and waiving criteria are currently lacking. Furthermore, we recommend that increasing attention is directed towards regulatory reliability and relevance of the information that is submitted by the registrants.

## 1. Introduction

Widespread use of nanomaterials (NMs) and their inevitable release to the environment have led to concerns for the environment and human health. NMs possess unique properties, afforded by their high surface area-to-volume ratio<sup>1</sup> and by engineering the material characteristics at the nanoscale such as size, shape and surface coating to fit a specific purpose.<sup>2</sup> This can change properties such as reactivity, surface composition, and dispersion stability, causing the nanomaterials to behave differently from their macroscale material counterparts.<sup>1-3</sup>

Several health and environmental legislations exist in Europe with the purpose, among others, to secure safe use and production of chemicals and consumer products. The need for considering

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37 nano-specific properties in these regulations has received growing attention and implementation of  
38 regulation with nano-specific provisions has increased substantially in recent years. In April 2018,  
39 the European Union adopted Annex revisions<sup>4</sup> in its chemical legislation called Registration,  
40 Evaluation, Authorisation and Restriction of Chemicals (REACH).<sup>5</sup> REACH applies to NMs and  
41 operates with tonnage-triggered data requirements meaning that the higher the annual production  
42 volume per producer, the more chemical safety information and data the registration has to provide.  
43 The tonnage-triggered data requirements are laid down in the annexes with a lowest tonnage trigger  
44 of one tonne per year. The total tonnage of a substance, including a NM, placed on the market is the  
45 sum of the individual tonnages per registrant. With the revisions of REACH, a comprehensive set of  
46 specific requirements for NMs was introduced. The new requirements, which have been in force since  
47 January 1, 2020, apply to manufacturers, importers and downstream users of NMs and establish  
48 specific rules regarding registration and chemical safety assessment (referred to as 'safety assessment'  
49 hereafter) of NMs. A NM must be registered in order to be placed on the market if the total yearly  
50 amount of the substance, including both NMs and potential bulk form, is above one tonne.<sup>4,6</sup> The  
51 requirement to complete a safety assessment is triggered for substances in quantities of more than 10  
52 tonnes.<sup>5</sup>

53 In order to comply with the new registration and safety assessment requirements, a  
54 comprehensive set of information on the NMs must be provided. This information includes among  
55 others information on physical and chemical properties of NMs (such as size, morphology and  
56 dissolution rates), environmental fate as well as their toxic potency.

57 At the initial deadline (January 1, 2020), the European Chemicals Agency (ECHA) had only  
58 received 50 unique registrations covering 16 substances.<sup>7</sup> By February 2020, ECHA had merely  
59 received 95 unique registration updates covering 36 chemicals in nanoform corresponding to 10% of  
60 their expected number of registrations. Updates were received on among other titanium dioxide,  
61 multi-walled carbon nanotubes, cerium oxide and carbon black, but information on some 270  
62 substances was missing when compared to what ECHA had foreseen based on national inventories  
63 and an EU catalogue of NMs in cosmetics.<sup>8</sup> It has furthermore been reported that almost half of the  
64 submissions that were received originally failed the completeness check due to incomplete  
65 justifications for registering separate nanoforms as sets of similar nanoforms or for waiving certain  
66 information requirements.<sup>8</sup> This has since then been improved for all, but two registrations after the  
67 companies were given a four months deadline.<sup>9</sup> Seven months after the original deadline for  
68 submissions had ECHA received 136 unique registration updates covering 52 chemicals. This is  
69 estimated to correspond to one-sixth of substances containing nanoforms.<sup>9</sup> There is currently no  
70 public information available on how many of the registrations are for substances produced in  
71 volumes of 100 tonnes or above per year. Interestingly, ECHA has not received any testing proposals  
72 for the Annex IX and X endpoints that are included in the REACH Annex revisions, including  
73 endpoints such as bioaccumulation in aquatic species and effects on terrestrial organisms. The  
74 tonnage triggers for the information requirements in Annex IX and X are 100 and 1000 tonnes or  
75 more, respectively. According to ECHA, it remains to be seen if registrants are currently mainly  
76 focusing on their specific nanoform characterisation and not (yet) on the hazard information.<sup>8</sup>

77 Application of conventional methods to test these parameters is often challenged by the specific  
78 properties of NMs and their non-equilibrium system-dependent behaviours. This has imposed the  
79 need for new or modified methods.<sup>10</sup>

80 There is currently no full overview of the availability and suitability of methods needed to fulfil  
81 the new nano-specific requirements in REACH. This places NM producers and users in a challenging  
82 situation as articulated by the European Chemical Industry Council (Cefic). Cefic is the branch  
83 organisation of the European chemical industry and consists of a network of 600 companies across  
84 90 different sectors. In a recent letter,<sup>11</sup> Cefic argues that there is a lack of fully developed test methods  
85 to fulfil the requirements. Together with the European non-ferrous metals association, Eurometaux,  
86 Cefic also pointed to concerns about business confidentiality and delays in the provisions of necessary  
87 IT tools and guidance provided by ECHA. As a consequence, Cefic and Eurometaux call for more  
88 time and flexibility for registrants to perform updates. They suggested to waive data generation for  
89 information requirements without associated test methods, or standards, until these become

90 available and furthermore enforcement could direct Cefic to contact companies that have not yet  
91 updated their existing dossiers.<sup>12</sup> EU Member States have also pointed at the absence of available test  
92 methods for certain endpoints as well as lack of laboratory capacity and short timeframe allowed for  
93 compliance. On the other hand, representatives from two NGOs, the European Environment Bureau  
94 and Center for International Environmental Law, have called for ECHA to take a tougher stand and  
95 deny the NM market access unless a minimum set of data is submitted. They argue that the lack of  
96 guidance does not help to explain the lack of registrations.<sup>12</sup>

97 ECHA has acknowledged the challenges that industry and registrants face to generate the  
98 necessary data and the length of time needed to revise the available OECD test guidelines (TGs), on  
99 which the agency's own guidance relies.<sup>7</sup> ECHA has publicly stated that it has adopted a temporary  
100 approach for endpoints where an internationally agreed test method does not exist and states that  
101 the lack of certain test methods should not be a hurdle in the registration process. It has also been  
102 reported that ECHA has postponed the publication of updated guidance on characterisation of  
103 human health and environmental endpoints to ensure that internationally recognised test methods  
104 are available.<sup>7</sup> Updates of ECHA's existing guidance made for NM substance identification and  
105 chemical safety assessment, published in 2017,<sup>6,13</sup> have been postponed and are expected in August  
106 2021 and September 2022 for human health endpoints and environmental endpoints, respectively.  
107 The stakeholder consultation is planned for the first quarter of 2021 for human endpoints and for the  
108 second quarter of 2022 for environmental endpoints.<sup>7</sup> Until then, the European Union Observatory  
109 for NM (EUON) hosted by ECHA has prepared an updated list of test guidelines and ongoing test  
110 guideline developments relevant for meeting the information requirements of the REACH Annexes.<sup>14</sup>

111 This study aims to assess the degree of availability and suitability of methods needed to comply  
112 with the new regulatory provisions on NMs for physico-chemical characterisation and environmental  
113 fate and effect assessment. The latter is especially interesting to study as updates to the official  
114 guidance have been called for very recently by experts from the German Environment Agency (UBA)  
115 and the Dutch National Institute for Public Health and the Environment (RIVM).<sup>15,16</sup> UBA and RIVM  
116 argued that significant uncertainties will remain as long as standardised TGs, guidance and  
117 monitoring data are lacking and call for the use of an additional safety factor to account for nano-  
118 related uncertainties.<sup>15</sup> Many information requirements include criteria that either impose additional  
119 requirements or waive requirements, in case they are fulfilled. The availability of methods needed to  
120 evaluate these criteria will also be assessed. Based on our analysis of methodological gaps,  
121 recommendations will be provided on how to prioritise future efforts to assist NM registrants meet  
122 their new legal obligations.  
123

## 124 **2. Materials and Methods**

### 125 *2.1 Literature review*

126 A literature review was conducted to identify available methods applicable for gathering the  
127 information according to data requirements for registration of nanoforms under REACH. The same  
128 procedure was carried out for the criteria included in the information requirements (criteria to impose  
129 additional requirements or waiving criteria). Web of Science was used to screen for methods in  
130 published literature. For every synonym of nanomaterials identified, searches were conducted with  
131 keywords related to methods (i.e. methods, tools, assessments, measurements and evaluations)  
132 combined with keywords related to the individual information requirements and waiving criteria  
133 (dissolution, biodegradation and particle size distribution etc.). Backward and forward reference  
134 searches were conducted for relevant references identified. Targeted searches for methods  
135 established in TGs, standards or other type of guidance were carried out within the frameworks of  
136 Organisation for Economic Co-operation and Development (OECD), the International Organization  
137 for Standardization (ISO) and European Chemicals Agency (ECHA) and their respective databases  
138 and catalogues.

### 139 *2.2 Categorisation of methods*

140

141 All identified methods relevant to information and data requirements for registration of  
142 nanoforms under REACH were evaluated. Each method was assigned to one of the following  
143 categories: Internationally accepted test guideline or standard (TGS), Internationally accepted test  
144 guideline or standard under development (TGSUD), Established as standard method in the scientific  
145 literature (SCI), Other method and/or more research needed (O) or No method (N). For information  
146 and data requirements where no methods were identified, the method category No method (N) was  
147 applied. Methods established as TGS (e.g. an OECD TG or ISO standard)) are considered the most  
148 important from a regulatory perspective as these are the fundament of current risk assessment  
149 procedures and of ECHA's guidance on chemical safety assessment. According to EUON, TGs are  
150 needed to support the regulatory testing of nanomaterials and should assist to ensure that tests are  
151 done uniformly across different labs and are delivering relevant and reliable data.<sup>14</sup> OECD develops  
152 both OECD TGs and OECD GDs, which are different types of documents for regulatory testing of  
153 chemicals. The OECD TGs are internationally recognised test methods and their results fall under  
154 OECD's system of Mutual Acceptance of Data (MAD). OECD GDs are not part of the MAD system.  
155 As far as possible the OECD test guideline programme develops TGs and GDs may be developed if  
156 e.g. particular groups of materials need additional explanation. In 2006, OECD established a Working  
157 Party on Manufactured Nanomaterials (WPMN), which aims at discussing regulatory aspects  
158 regarding nano-safety and assessing whether existing TGs can be applied to NMs, and exploring the  
159 need for new TGs that would be nano-specific. The development of new OECD TGs is built on  
160 knowledge from available research and regulatory requirements.<sup>17</sup> These TGs describe how to test  
161 and report on potential effects of chemicals, including among others, their physical-chemical  
162 properties and environmental fate and behavior. ISO develops international standards within a wide  
163 range of areas, including chemical technology and environmental management, some of which  
164 overlap with the OECD TGs e.g. on ecotoxicity of chemicals. ISO standards are developed by  
165 technical committees (TCs) consisting of groups of experts representing the relevant sectors.<sup>18</sup> For  
166 nanomaterials the relevant committee is ISO/TC 229 on Nanotechnologies. In addition to  
167 international standards, ISO develops technical specifications (TSs) and technical reports (TRs) and  
168 these do not account for TGs in this analysis. A TS entails work that is still under development and  
169 can be provided for immediate use and/or a tool to obtain feedback until it may eventually be  
170 republished as an international standard. The information provided in a TR can e.g. include data  
171 obtained from surveys or information of the perceived "state of the art" in an area.<sup>19</sup> ECHA manages  
172 the technical and administrative aspects of REACH and has developed a range of guidance to assist  
173 manufacturers and importers of chemicals in complying with REACH. Most of this guidance refer to  
174 OECD TGs and ISO standards, but when not available also on the scientific literature.<sup>6,13</sup> The method  
175 category 'Established as standard method in the scientific literature' entails methods that are not  
176 standardised methods in the sense that OECD TGs and/or ISO standards exist, but which often are  
177 referred to and used in the scientific literature to an extent where it is considered a minimum  
178 reporting requirement for scientific publications.<sup>20,21</sup> Methods that also belong to this method  
179 category are those for which OECD GDs or ISO TRs or TSs exist. Methods that are under development  
180 which are only available to a limited number of pioneering research groups or that have only been  
181 discussed theoretically in the scientific literature fall into the category 'Other method and/or more  
182 research needed'. Only one method category are assigned to each information requirement or criteria.  
183 In situations where more than one method are available for one information requirement or criteria,  
184 the highest ranked method category applies. The method categories are ranked highest to lowest: TG,  
185 TGUD, S, O and N. Methods were evaluated according to their availability and suitability. For  
186 evaluating availability, criteria included consideration of whether methods are public accessible/free  
187 of charge as well as whether certain technology is needed for its application and how available this  
188 technology is. Suitability was assessed by considering if the identified methods are applicable to  
189 nanomaterials or whether noteworthy limitations exist.

### 190 3. Results

191 For all the information requirements listed in the REACH Annexes,<sup>4</sup> we gathered information about  
192 the availability of methods, protocols and guidelines from OECD, ISO and ECHA and their respective  
193 databases and catalogues as well as the scientific literature. Table 1 provides an example of  
194 information compiled for the first information requirement, namely 7.7 Solubility (and dissolution  
195 rate). A full overview of all information collected for each information requirement is given in  
196 Electronic Supplementary Material 1 and 2.

197 **Table 1.** Example of information collected on the availability of methods, protocols and guidelines for the information requirement 7.7 Solubility (and dissolution  
 198 rate) and its waiving criteria and the highest-ranked category of identified methods needed to acquire/fulfill the information requirement and its waiving criteria  
 199 set by the European chemicals legislation REACH for nanomaterials (NMs). Details for the full overview is given in Electronic Supplementary Material 1. TGS:  
 200 Internationally accepted test guideline or standard; TGSUD: Internationally accepted test guideline under development; SCI: Established as standard method in  
 201 scientific literature; O: Other method and/or more research needed; N: No method identified. The method categories are ranked highest to lowest: TGS, TGSUD,  
 202 SCI, O and N. Other abbreviations: TR: technical report; GD: guidance document; Organisation for Economic Co-operation and Development (OECD); ISO:  
 203 International Organization for Standardization; ECHA: the European Chemicals Agency. WNT: Working Group of the National Coordinators for the Test  
 204 Guidelines Programme.

Information requirement	Waiving criteria	Methods, protocols and guidance	Highest method category
7.7 Solubility (and dissolution rate)		<ul style="list-style-type: none"> <li>• OECD WNT Project 3.10: New TG on dissolution rate of NMs in aquatic environment ongoing.<sup>22</sup></li> <li>• OECD WNT Project 1.5: GD on determination of solubility and dissolution rate of NMs in water and relevant synthetic biological media ongoing for new guidance document/test guideline.<sup>22</sup></li> <li>• GD 318 for the testing of dissolution and dispersion stability of NMs, and the use of the data for further environmental testing and assessment.<sup>23</sup> The GD lists the following methods:               <ol style="list-style-type: none"> <li>1. Batch test using Centrifugal ultrafiltration (not recommended ultracentrifugation or dialysis)</li> <li>2. Dynamic testing of dissolution rates based on Koltermann-Jülly et al.<sup>24</sup></li> </ol> </li> <li>• Partially covered in ECHA Guidance.<sup>13,14</sup></li> <li>• OECD TG 105<sup>25</sup>: not adapted to NMs specifically</li> <li>• ISO/TR 19057<sup>26</sup>: adapted to NMs</li> <li>• OECD GD 29 on Transformation/ Dissolution of Metals and Metal Compounds in Aqueous Media<sup>27</sup> is applicable for some NMs. E.g., Wasmuth et al.<sup>28</sup> applied it successfully to silver NMs.</li> <li>• Multiparameter matrix methods may be used to study dissolution and solubility. E.g., Xiao et al.<sup>29</sup> performed a multifactorial design for the water chemistry (based on OECD-TG-318 matrix of parameters: natural organic matter, ionic strength and pH).</li> <li>• Study of dissolution of metal NMs environmental (freshwater) conditions.<sup>30,31</sup></li> </ul>	TGSUD

(7.7.)  
Hydrolytically  
stability or  
readily  
oxidisable

- OECD Project 3.10 (New TG on dissolution rates of NMs in aquatic environments) includes a screening test to assess whether they fall into the category of rapidly dissolving NMs.<sup>22</sup>
- \*\*

TGSUD

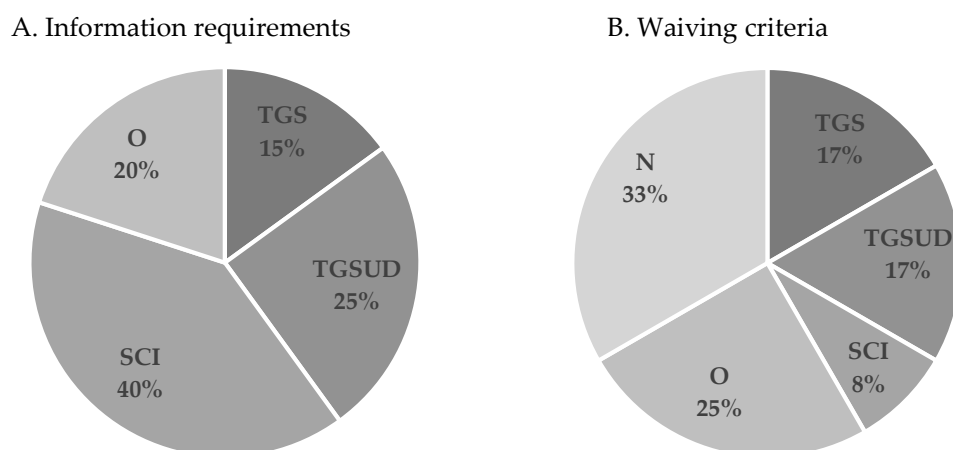
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205 \*\* Hydrolytically NMs are in this context considered unstable or readily oxidisable or rapidly dissolving (half-life less than 12h).



206 3.1. Information requirements

207 In total, there are 20 additional, nano-specific information requirements for which methods are  
208 needed in order to register substances that are (also) nanoforms in REACH. All the identified  
209 methods were categorised as described above. Figure 1 shows the distribution of methods categories  
210 for methods to acquire/fulfill information requirements of REACH regulation on NMs.  
211 Internationally accepted TGs or standards are available (see Figure 1A) for 15% (3 out of 20) of the  
212 information requirements. This is the case for information requirements 'Further information on  
213 physicochemical properties', 'Simulation testing on ultimate degradation in surface water' and  
214 'Hydrolysis as function of pH', for which OECD TGs exist.



215

216 **Figure 1.** Distribution of method categories for methods, protocols and guidance identified to acquire/fulfill information  
217 requirements (A) and to assess waiving criteria for information requirements (B) in the REACH Annexes on nanomaterials.  
218 Method categories represents the highest-ranked method for each information requirement and waiving criteria, where the  
219 ranking from highest to lowest are: TGS, TGSUD, SCI, O and N. TGS: Internationally accepted test guideline; TGSUD:  
220 Internationally accepted test guideline under development; SCI: Established as standard method in scientific literature; O:  
221 Other method and/or more research needed; N: No method identified.

222

223 For 25% (5 out of 20) of the information requirements, we identified methods that are under  
224 development into TGs or standards. This was especially the case for methods related to solubility  
225 (and dissolution rate) and dustiness, but also aspects of particle characterisation such as particle size  
226 distribution, shape and specific surface area (see Table 2). Table 2 provides a summary on information  
227 gathered in the Electronic Supplementary Materials 1 and compiles methods, protocols and guidance  
228 that we have been able to identify for each information requirements together with our categorisation  
229 of the best-ranked identified method for each requirement.

230 ECHA directly cites or considers the state-of-the-art within the scientific literature which are  
231 related to the new information requirements for NMs. Our literature review shows that 40% (8 out  
232 of 20 information requirements) include modified standard methods or advices to best practice that  
233 are available in the scientific literature or in OECD GDs, ECHA guidance or TRs/TS's by ISO. These  
234 include methods related to growth inhibition studies on aquatic plants, short-term toxicity testing on  
235 fish and invertebrates, and activated sludge respiration inhibition testing, for which methods are  
236 presented in OECD GD 317,<sup>32</sup> as well as methods to assess bioaccumulation in aquatic species and  
237 effects on terrestrial organisms. Included in this category (S) is also the information requirement  
238 related to description of surface functionalisation or treatment, for which e.g. ECHA guidance<sup>6</sup> exists,  
239 and characterisation of particle aggregation and agglomeration, for which certain imaging methods  
240 are established (see Table 2).

241 Four out of the 20 information requirements fall under the method category 'Other method  
242 and/or more research is needed (O)'. These are e.g. methods related to assessing  
243 adsorption/desorption and the partition coefficient n-octanol/water (see Table 2), for which ECHA

244 has announced that methodological guidance is partially covered by guidance released on NM  
245 substance identification and chemical safety assessment.<sup>6,13</sup> Partition coefficient n-octanol/water is not  
246 considered applicable for particulate, insoluble NMs.<sup>33</sup> In the regulation it is stated that where the  
247 partition coefficient n-octanol/water is not applicable for a NM, dispersion stability shall be  
248 considered instead. For this, OECD TG 318<sup>34</sup> can be used. Another information requirement in the  
249 method category 'Other method and/or more research is needed (O)' is '9.2 Degradation'. The OECD  
250 Working Group of the National Coordinators for the Test Guidelines Programme (WNT) project 3.16  
251 is working on a GD addressing abiotic transformations of NMs in environmental media.<sup>22</sup> ECHA  
252 states that the guidance is likely to be available in 2023 or 2024 and that "*It may be possible to conduct*  
253 *a qualitative assessment in the meantime*".<sup>14</sup>

254 **Table 2.** The 20 additional, nano-specific information requirements for registration of nanomaterials (NMs) in the European chemicals legislation (REACH)<sup>4</sup> and  
 255 categories of identified highest-ranked method needed to acquire/fulfill the information requirements. TGS: Internationally accepted test guideline or standard;  
 256 TGSUD: Internationally accepted test guideline or standard under development; SCI: Established as standard method in scientific literature; O: Other method  
 257 and/or more research needed; N: No method identified. The method categories are ranked highest to lowest: TGS, TGSUD, SCI, O and N. Other abbreviations:  
 258 TR: technical report; TS: technical report; GD: guidance document; Organisation for Economic Co-operation and Development (OECD); ISO: International  
 259 Organization for Standardization; ECHA: the European Chemicals Agency; WNT: Working Group of the National Coordinators for the Test Guidelines  
 260 Programme. Roman numerals in parentheses and italics refer to the REACH annex that an information requirement belong to (VII = Annex VII/7, etc.).

Information requirement	Highest method category	Methods, protocols and guidance identified
7.7 Solubility (and dissolution rate) (VII)	TGSUD	OECD WNT Project 3.10: TG under development <sup>22</sup> ; OECD WNT Project 1.5: GD under development <sup>22</sup> ; OECD GD 318 <sup>23</sup> ; ECHA Guidance R7.1a and R7.1b appendices** <sup>13</sup> ; OECD GD 29 <sup>27</sup> : applicable for some NMs; Multiparameter matrix methods <sup>e.g. 29,35</sup> ; Dissolution study of metal NMs environmental (freshwater) conditions. <sup>30,31</sup>
7.8 Partition coefficient n-octanol/ water (VII)	O	ECHA Guidance**. <sup>13,14</sup>
7.14bis Dustiness (VII)	TGSUD	OECD WNT Project 1.8: TG under development <sup>22</sup> ; Rotating drum method, continuous drop method and small rotating drum method, <sup>33,36</sup> for which British standards exist: EN 15051-1, <sup>37</sup> 15051-2 <sup>38</sup> and EN 15051-3. <sup>39</sup>
9.1.1 Short-term toxicity testing on invertebrates (VII)	SCI*	ISO/TS 20787:2017 <sup>40</sup> ; OECD GD 317. <sup>32</sup>
9.1.2 Growth inhibition study aquatic plants (VII)	SCI*	OECD GD 317. <sup>32</sup>
7.14ter Further information on physico-chemical properties (VIII)	TGS	OECD decision tree <sup>14</sup> ; OECD framework on physical chemical characterisation <sup>14</sup> ; OECD WNT Project 1.7: TG under development <sup>22</sup> ; ISO/TR 11360:2010. <sup>14,41</sup>
9.1.3 Short-term toxicity testing on fish (VIII)	SCI*	OECD GD 317. <sup>32</sup>
9.1.4 Activated sludge respiration inhibition testing (VIII)	SCI*	OECD GD 317. <sup>32</sup>
9.2 Degradation (VIII)	O	OECD WNT 3.16 Project: GD under development. <sup>22</sup> Qualitative assessment may be possible in the meantime. <sup>14</sup>
9.2.2.1 Hydrolysis as function of pH (VIII)	TGS	OECD TG 318. <sup>34</sup>
9.3.1 Adsorption/desorption screening (VIII)	O*	ECHA Guidance 2017** <sup>13,14</sup> ; Dye staining to measure hydrophobicity of NMs in aquatic environments. <sup>42</sup>

9.2.1.2 Simulation testing on ultimate degradation in surface water (IX)	TGS	OECD TG 318. <sup>34</sup>
9.3.2 Bioaccumulation in aquatic species, preferably fish (IX)	SCI	ICP-MS or AAS for analysis of NMs in dissolved tissue of aquatic organisms <sup>e.g. 43-45</sup> ; In vivo and in vitro methods for acellular dissolution in physiological fluids <sup>e.g. 36</sup> ; OECD WNT Project 3.12: GD under development. <sup>22</sup>
9.3.3 Further information on adsorption desorption depending on the results of the study required in Annex VIII (IX)	O	ECHA Guidance <sup>** 13,14</sup> ; Dye staining to measure hydrophobicity of NMs in aquatic environments. <sup>42</sup>
9.4 Effects on terrestrial organisms (IX)	SCI	OECD GD 317 <sup>32</sup> may be updated for terrestrial organisms <sup>14</sup> ; Equilibrium partitioning method may be applied, if scientifically justified <sup>4</sup> ; Proposal on OECD TG 312 <sup>46</sup> as alternative to equilibrium partitioning method <sup>47,48,13</sup> ; Draft OECD GD on testing nanomaterial behaviour in soils using OECD TG 312 under development <sup>14</sup> ; OECD TG 303A <sup>49</sup> also proposed as an alternative approach to predict sorption of NMs.
2.4.2 Number based particle size distribution with indication of the number fraction of constituent particles in the size range within 1 nm – 100 nm. (VI)	TGSUD	OECD WNT project 1.4: TG under development <sup>22</sup> ; ISO/TS 19590:2017 <sup>50</sup> ; ISO 22412:2017 <sup>51</sup> applies, with certain restrictions <sup>33</sup> ; ISO/TS 21362:2018 <sup>52</sup> ; Imaging methods, e.g. electron microscopy <sup>53</sup> ; Nanoparticle Tracking Analysis (NTA). <sup>54</sup>
2.4.3 Description of surface functionalisation or treatment and identification of each agent including IUPAC name and CAS or EC number. (VI)	SCI*	ECHA Guidance, <sup>6</sup> including analytical techniques (e.g. IR, NMR, TGA, ICP-MS, XRF, XPS, EDX, GC-MS, MALDI-TOF, etc.), NANoREG <sup>55</sup> and ISO TR 14187:2011. <sup>56</sup> Revised version: ISO/TR 14187:2020. <sup>57</sup>
2.4.4 Shape, aspect ratio and other morphological characterisation: crystallinity, information on assembly structure (VI)	TGSUD	OECD WNT Project 1.4: TG under development <sup>22</sup> ; Protocols from research projects and/or standard methods and/or scientific literature available for determination of crystallinity/assembly structure <sup>14</sup> ; Electron microscopy (EM) for determination of shape. <sup>33</sup>
2.4.5 Surface area (specific surface area by volume, specific surface area by mass or both) (VI)	TGSUD	OECD WNT Project 1.3: TG under development <sup>22</sup> ; ISO/TR 14187:2011 <sup>56,57</sup> provides some input to relevant information; ISO 9277:2010 <sup>58</sup> applies, with certain restrictions; Determination of the specific surface area of solids by gas adsorption – BET method, with restrictions. <sup>33</sup>
5.2.3 Characterisation the particle aggregation and the agglomeration (I)	SCI	Certain imaging methods, such as electron microscopy <sup>53,59</sup> ; Electron microscopy image and transmission electron topography <sup>60,61</sup> ; Draft OECD TG on Agglomeration Behaviour of

Nanomaterials in Different aquatic Media<sup>62</sup>; Development of a new OECD TG or GD on determination of aggregation/agglomeration status of NMs is under consideration<sup>33</sup>; Electron microscopy image and transmission electron topography image.<sup>60,61,63</sup>

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261 \* Method available through ECHA guidance, OECD GDs or ISO TSs/TRs

262 \*\* Information requirement only partially covered by ECHA Guidance.<sup>11</sup>

264 3.2. *Waiving criteria*

265 A number of waiving criteria are listed for many of the new nano-specific information  
266 requirements listed in the REACH Annexes.<sup>4</sup> This means that if these criteria are fulfilled for a given  
267 information requirement, then the registrant does not have to provide information on this given  
268 endpoint. Figure 1B shows the distribution of methods categories for methods needed to assess the  
269 waiving criteria.

270 Our study shows that 12 different waiving criteria are contained in the REACH information  
271 requirements for NMs. 17% (2 out of 12) of the waiving criteria were supported by methods in the  
272 form of internationally accepted TGs or standards, including methods related to biodegradability  
273 and decomposition of the NM (see Table 3). Table 3 summarises the collected information (see  
274 Electronic Supplementary Material 1) and includes methods, protocols and guidance that we have  
275 been able to identify for each waiving criterion and our categorisation of the best-ranked identified  
276 method for assessing each criterion. For another 17% of the waiving criteria, methods in the form of  
277 internationally accepted TGs or standards are currently under development. This applies to methods  
278 to inform waiving criteria on solubility and hydrolytically stability/readily oxidisable.

279 For one waiving criterion, i.e. 'The substance has low potential for bioaccumulation (for instance  
280 a  $\log K_{ow} \leq 3$ '), the method category SCI was assigned. The methods identified in the scientific  
281 literature to assess the criterion include ICP-MS or AAS for analysis of NMs in dissolved tissue of  
282 aquatic organisms.<sup>e.g. 43-45</sup>

283 Other methods/more research are needed for 25% of the waiving criteria (3 out of 12). That is  
284 e.g. the criterion describing that the information requirement can be waived for inorganic NMs. The  
285 criterion related to the likelihood/potential of the substance to cross biological membranes also falls  
286 into this category (O). No methods were identified that can sufficiently assess such  
287 likelihoods/potentials. However, a list of methods to partly help inform this criterion were gathered  
288 (see Table 3).

289 No methods were identified that can support whether the criteria can be waived for a third (4  
290 out of 12) of the waiving criteria. This was especially the case for the criteria that rely on an assessment  
291 of specific exposure scenarios of a NM of concern. Examples of this kind of waiving criteria include  
292 '*exposure to granular form of the substance during its life-cycle can be excluded*' and '*direct and indirect*  
293 '*exposure of the aquatic compartment is unlikely*'.

294 Certain information requirements are linked to criteria that can impose additional information  
295 requirements to the substance of concern. Table 4 provides an overview of these criteria and  
296 requirements and the identified methods needed to evaluate/fulfill them.

297 **Table 3.** Waiving criteria for information requirements for registration of nanomaterials (NMs) in the European chemicals legislation (REACH) and category of  
 298 identified highest-ranked method needed to assess if waiving criteria are triggered. TGS: Internationally accepted test guideline or standard; TGSUD:  
 299 Internationally accepted test guideline or standard under development; SCI: Established as standard method in scientific literature; O: Other method and/or more  
 300 research needed; N: No method identified. The method categories are ranked highest to lowest: TGSS, TGUD, SCI, O and N. Other abbreviations: TR: technical  
 301 report; TS: technical report; GD: guidance document; Organisation for Economic Co-operation and Development (OECD); ISO: International Organization for  
 302 Standardization; ECHA: the European Chemicals Agency; WNT: Working Group of the National Coordinators for the Test Guidelines Programme.

Waiving criteria	Highest method category	Methods, protocols and guidance identified
(7.7) Hydrolytically stability or readily oxidisable*	TGSUD	OECD WNT Project 3.10: TG under development. <sup>22</sup>
(7.8) The substance is inorganic	O	<i>No method needed.</i>
(7.14bis) Exposure to granular form of the substance during its life-cycle can be excluded	N	<i>No method identified.</i>
(9.1.1; 9.1.2; 9.1.3; 9.1.4; 9.2.2.1; 9.2.1.2) The substance is highly insoluble in water**	TGSUD	OECD WNT Project 3.10: TG under development <sup>22</sup> ; OECD WNT Project 1.5: GD under development. <sup>22</sup>
(9.1.1; 9.1.2; 9.1.3; 9.1.4) The substance is unlikely/has low potential to cross biological membranes	O	Flow-cytometry, microscopy, ICP-MS and TEM for cellular uptake <sup>61</sup> ; In vitro assay(s) for cytotoxicity and cytokine induction <sup>64,65</sup> ; Biokinetics-oriented tests for capability of cell penetration. <sup>66,67</sup>
(9.2.2.1; 9.2.1.2; 9.1.4) The substance is readily biodegradable	TGS	OECD TG 301A-F. <sup>68</sup>
(9.1.4) The applied test concentrations are in the range of concentrations that can be expected in the influent of a sewage treatment plant	N	<i>No method identified.</i>
(9.3.1) Based on physicochemical properties, the substance is expected to have a low potential for adsorption (e.g. the substance has a low octanol-water partition coefficient)	O	ECHA Guidance <sup>***</sup> 13,14; Dye staining to measure hydrophobicity. <sup>42</sup>
(9.3.2) The substance has low potential for bioaccumulation (for instance a log $K_{ow} \leq 3$ )	SCI	ICP-MS or AAS for analysis of NMs in dissolved tissue of aquatic organisms <sup>e.g. 43-45</sup> ; In vivo and in vitro methods for acellular dissolution in physiological fluids <sup>e.g. 36</sup> ; OECD WNT Project 3.12: GD under development. <sup>22</sup>

(9.3.1; 9.3.3) The substance and its relevant degradation products decompose rapidly	TGS	OECD 301A-F. <sup>68</sup>
(9.3.2) Direct and indirect exposure of the aquatic compartment is unlikely	N	<i>No method identified</i>
(9.4) Direct and indirect exposure of the soil compartment is unlikely	N	<i>No method identified</i>

303 \* Hydrolytically NMs are in this context considered unstable or readily oxidisable or rapidly dissolving (half-life less than 12h); \*\* For nanoforms, the study may  
304 not be waived on the basis of high insolubility in water alone; \*\*\* Information requirement only partially covered by ECHA Guidance.<sup>11</sup>

305

306 **Table 4.** Additional information requirements and the criteria for triggering the additional requirements for registration of nanoforms in REACH and category of  
307 identified highest-ranked methods needed to acquire/fulfill information requirements and associated criteria. TGS: Internationally accepted test guideline or  
308 standard; TGSUD: Internationally accepted test guideline or standard under development; SCI: Established as standard method in scientific literature; O: Other  
309 method and/or more research needed; N: No method identified. For further details, see Electronic Supplementary Material B. The method categories are ranked  
310 highest to lowest: TGS, TGSUD, SCI, O and N. Other abbreviations: TR: technical report; TS: technical report; GD: guidance document; Organisation for Economic  
311 Co-operation and Development (OECD); ISO: International Organization for Standardization; ECHA: the European Chemicals Agency; WNT: Working Group of  
312 the National Coordinators for the Test Guidelines Programme.

Criteria for additional information requirements	Additional information requirements	Highest method category	Methods, protocols and guidance identified
(7.7.) The substance appears “insoluble” in water		TGSUD	OECD WNT Project 1.5 and 3.10 <sup>22</sup> ; OECD GD 318. <sup>23</sup>
	(7.7) Limit test up to the detection limit of the analytical method shall be performed. For nanoforms the potential confounding effect of dispersion shall be assessed when conducting the study.	TGS	OECD TG 318 <sup>34</sup> addresses dispersion stability of nanomaterials in simulated environmental media using multi-parameter matrix and analysis by DLS, ICP-MS/OES and AAS techniques.



(9.1.1) The substance is poorly water soluble, or for nanoforms if they have low dissolution rate in the relevant test media.

TGSUD

OECD WNT Project 3.10 TG under development<sup>22</sup>; OECD WNT Project 1.5: GD under development<sup>22</sup>; OECD GD 318.<sup>22</sup>

(9.1.1) The long-term aquatic toxicity study on Daphnia (Annex IX, section 9.1.5.) shall be considered

TGS

OECD TG 211: Daphnia magna Reproduction Test.<sup>69</sup>

(7.14ter) Indication that specific additional particle properties significantly influence the hazard of or the exposure to those nanoforms

Essential to determine the kinetics of changes in surface reactivity/composition, particle stability/mobility in solution etc. Surface speciation and chemical speciation prediction in solution is essential to assess.<sup>31</sup>

(8.8.1) For nanoforms without high dissolution rate in biological media

TGSUD

OECD WNT Project 3.10: TG under development<sup>22</sup>; OECD WNT Project 1.5: GD under development<sup>22</sup>; OECD GD 318<sup>23</sup>; ECHA Guidance R7.1a and R7.1b appendices\*<sup>13</sup>; OECD GD 29<sup>27</sup>: applicable for some NMs; Multiparameter matrix methods<sup>e.g. 29,35</sup>; Dissolution study of metal NMs environmental (freshwater) conditions.<sup>30,31</sup>

8.8.1) A toxicokinetics study shall be proposed by the registrant or may be required by the Agency in accordance with Article 40

(9.1.3) The chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms.

	(9.1.3) Long-term aquatic toxicity testing as described in Annex IX shall be considered.		
(9.1.3) The substance is poorly water soluble, or for nanoforms if they have low dissolution rate in the relevant test media.		TGSUD	OECD WNT Project 3.10: TG under development <sup>22</sup> ; OECD WNT Project 1.5: GD under development <sup>22</sup> ; OECD GD 318 <sup>23</sup> .
	(9.1.3) The long-term aquatic toxicity study on fish (Annex IX, Section 9.1.6) shall be considered	TGS	OECD TG 204 <sup>70</sup> .
(9.2) Nanoforms that are not soluble, nor have high dissolution rate		TGSUD	OECD WNT Project 3.10: TG under development <sup>22</sup> ; OECD WNT Project 1.5: GD under development <sup>22</sup> ; OECD GD 318 <sup>23</sup> .
	(9.2) Morphological transformation (e.g. irreversible changes in particle size, shape and surface properties, loss of coating)	SCI	Band gap measured by UV-vis-NIR absorption measurements; probe force microscopy; soft X-ray methods. <sup>e.g. 36</sup> ; Redox potential measurements. <sup>e.g. 71,72</sup>
	(9.2) Chemical transformation (e.g. oxidation, reduction)	SCI	Combining TEM with energy-dispersive X-ray spectroscopy (EDX) for elemental composition of the nanoparticles <sup>61,73</sup> ; Attenuated Total Reflection Fourier transform infrared Spectroscopy (ATR-FTIR) <sup>74</sup> ; Atomic force microscopy (AFM) – changes in particle morphology and size <sup>e.g. 75</sup> ; Zeta potential measurement <sup>76</sup> and reporting <sup>77</sup> ; X-ray Absorption Spectroscopy. <sup>78</sup>
	(9.2) Other abiotic degradation (e.g. photolysis)		
(9.4) For substances that have a high potential to adsorb to soil		TGSUD	Spectroscopic tools like FTIR <sup>74</sup> and EXAFS <sup>79</sup> ; OECD TG 312 <sup>46</sup> is applicable for NMs <sup>13,47,48</sup> ; Retention determination of silver and

			cerium oxide nanomaterials in soils. <sup>80</sup> ; Draft OECD TG on Agglomeration Behaviour of Nanomaterials in Different aquatic Media. <sup>62</sup>
	(9.4) The registrant shall consider long- term toxicity testing instead of short-term.	TGS	OECD TG 204. <sup>70</sup>
(9.4) For substances that are very persistent		O	OECD WNT 3.16 project: GD under development. <sup>22</sup>
	9.4) The registrant shall consider long- term toxicity testing instead of short-term.	TGS	OECD TG 204. <sup>70</sup>

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313 \* Information requirement only partially covered by ECHA Guidance.<sup>11</sup>

## 314 4. Discussion

### 315 4.1. Information requirements

316 According to our findings, almost 80% of the information requirements related to physico-chemical  
317 properties and environmental endpoints of NMs in the revised REACH Annexes are supported by  
318 methods that are established as internationally accepted TGs or standards (15%), under development  
319 to be established as internationally accepted TGs or standards (25%) or established in the scientific  
320 literature (40%). Overall, ECHA guidance and OECD GDs are in place where TGs or standards are  
321 missing as presented in a recent overview of guidelines and other guidance.<sup>14</sup> Key TGs and guidance  
322 include OECD TG 318<sup>34</sup>, OECD GD 317<sup>32</sup> and 318<sup>23</sup> and ECHA guidance released for NM substance  
323 identification and chemical safety assessment, especially the R7.1a and R7.1b appendices.<sup>13</sup> These  
324 documents as well as the huge work efforts made to reach these important milestones are recognised  
325 as a significant advancement in the regulatory field of NMs.

326 Despite the progress made to develop nano-specific methods and guidance, the application of  
327 these to some NMs may still pose a number of challenges. For example, this is the case for the  
328 regulation information requirement 9.4 on effects on terrestrial organisms, for which ECHA notes  
329 that OECD GD 317<sup>32</sup> may be updated to help registrants fulfill the requirement.<sup>14</sup> While this awaits,  
330 it is stated that: *“In the absence of toxicity data for soil organisms, the equilibrium partitioning method may*  
331 *be applied to assess the hazard to soil organisms.”*<sup>4</sup> However, the suitability and relevance of partitioning  
332 measures for NMs have been rejected in the scientific literature.<sup>81,82</sup> This is also recognised in the  
333 regulation and it is clarified that where the equilibrium partitioning method is applied to nanoforms,  
334 this shall be scientifically justified. Based on the current scientific knowledge, it seems unlikely that  
335 it will be possible to provide such justification. The issue of applying partitioning measures to NMs  
336 also applies to the closely related information requirement: partition coefficient n-octanol/water.  
337 According to ECHA,<sup>14</sup> methods to support this information requirement are partially covered in the  
338 ECHA Guidance on registration and chemicals safety assessment of nanomaterials.<sup>13</sup> ECHA  
339 furthermore inform that *“Dispersion stability to be considered when  $K_{ow}$  is not applicable”* and refer to TG  
340 318: Dispersion Stability of Nanomaterials in Simulated Environmental Media.<sup>34</sup> While this method  
341 partly covers interaction with natural organic matter it does not cover the functionality changes in  
342 relation to e.g. changes in hydrophobicity<sup>42</sup> which would change the nanoparticle fate in the  
343 environment. For rapidly soluble NMs the same approach would apply as for conventional  
344 chemicals.<sup>33</sup> Instead, relevant information would be dissolution rate, according to the WPMN.<sup>17</sup>  
345 However, for solubility (and dissolution rate) the methods are under development and NM  
346 registrants are in the meantime referred to the OECD GD 318.<sup>23</sup> In Valsesia et al.<sup>83</sup> a method for  
347 quantifying surface hydrophobicity of NMs is proposed as an alternative method to octanol-water  
348 coefficient relevant information. Quantification is done by comparing NM binding affinity to  
349 specifically functionalised surfaces. Moreover, it should be highlighted that a work is ongoing in the  
350 OECD on a TG on determination of surface hydrophobicity of nanomaterials. This work is anticipated  
351 to be finalised in 2023.

352 For the information requirements related to adsorption/desorption in aquatic environments,  
353 methods are only partially covered in guidance (i.e. ECHA guidance). Current methods relies mainly  
354 on partition coefficients and separation techniques such as filtration and centrifugation. When using  
355 such methods the cut-off threshold has to be considered in order to correctly interpret the results  
356 obtained. This can be challenging especially when considering unstable suspensions where the  
357 particle size changes as a function of time. In general, the methods presented are mainly applicable  
358 to highly soluble and/or small stable NMs. Adsorption as a function of interaction with naturally  
359 occurring substances should be considered, as they have been shown to alter e.g. hydrophobicity and  
360 thus the potential adsorption/desorption.<sup>42</sup> Hydrophobicity of NMs in aquatic environments can be  
361 measured by use of e.g. dye staining.<sup>42</sup> It has been proposed that OECD TG 312 Leaching in Soil  
362 Columns<sup>42</sup> could provide for an alternative approach to the equilibrium partitioning method.<sup>13,47,48</sup>  
363 For this method, a draft OECD GD for NMs is under development.<sup>14</sup> OECD TG 303A on Particle

364 Attachment and Removal from Wastewater<sup>49</sup> can furthermore be used as an alternative approach to  
365 predict sorption of NMs.

366 It is very important to note that it is not always possible to establish univocally whether methods  
367 exist that would enable a registrant to meet a given information requirement. For instance, in some  
368 cases there might be methods for which TGs, standards or guidance exist that are only applicable to  
369 some forms of NMs. An example, the OECD GD 29<sup>27</sup> is only applicable to metal NMs. For certain  
370 information requirements, the guidance referred to are only partially applicable to NMs. ECHA  
371 Guidance R7.1a and R7.1b appendices<sup>13</sup> does for example only partially cover the information  
372 requirement '9.3.1 Adsorption/desorption'.

373

#### 374 4.2. *Waiving criteria*

375 For the proper functioning of the regulation, it is crucial that the waiving criteria are clearly  
376 defined and supported by the necessary methods to justify their activation. Our analysis shows that  
377 for slightly more than 40% of the waiving criteria, methods are either available as internationally  
378 accepted TGs or standards (17%), under development to be (17%) or established in the scientific  
379 literature (8%). But for a third of the waiving criteria, no specific methods are identified.

380 The majority of the waiving criteria for which no methods were identified, relates to criteria for  
381 certain exposure scenarios, i.e., if a certain exposure is unlikely to occur, the information requirement  
382 can be waived. This is the case if exposure of certain forms of the substance or the exposure to certain  
383 systems or environmental compartments are evaluated to be unlikely. Currently, no methods exist to  
384 fully document such evaluations. For environmental exposure, fate models such as SimpleBox4Nano,  
385 that predicts environmental concentrations of NMs in air, water, sediment and soil<sup>84,85</sup> and  
386 Probabilistic material flow analysis<sup>86</sup> may be informative.<sup>87</sup> Despite progressive development of such  
387 models, they cannot stand alone to justify "no exposure"/"low exposure". Such indications of 'no  
388 exposure"/"low exposure" should be carefully evaluated case-by-case, before accepting any waiving  
389 of regulatory requirements. Lessons learned from previous cases show that though it may not be  
390 intended, substances used in society will inevitably be found in the environment at some point in  
391 time.<sup>88</sup>

392 Certain waiving criteria rely on the octanol-water coefficient,  $K_{ow}$  and sets out that an  
393 information requirement can be waived e.g. if the  $K_{ow}$  is low or under a certain value. As it is  
394 mentioned earlier for the information requirements based on partitioning methods, these principles  
395 are not deemed scientifically applicable to NMs.<sup>81,82</sup> In contrast to the information requirement, no  
396 alternative guidance is provided on how to assess this endpoint.

397 For inorganic NMs the information requirement on  $K_{ow}$  can be waived. Challenges may arise for  
398 substances that consist of both organic and inorganic materials, such as inorganic particles with  
399 organic coatings. Organic coatings are widely used to stabilise inorganic NPs, such as silver NPs.<sup>89</sup>  
400 In addition to consider inorganic substances with organic coatings as one substances it might be  
401 viewed as a mixture of two substances. However, it is not clear from the regulation how these  
402 substances are to be classified. Furthermore, some organic NMs may act inorganically. E.g. carbon  
403 nanotubes, C60 and carbon black have been proven to show persistence despite their carbon-based  
404 structure.<sup>90</sup>

405 The ecotoxicological tests required by the regulation does not need to be conducted if there are  
406 mitigating factors indicating that aquatic toxicity (for tests on invertebrates, algae and fish) and  
407 microbial toxicity (for activated sludge respiration inhibition testing) are unlikely to occur. This is for  
408 instance the case if the substance of concern is "highly insoluble in water". A new nano-specific  
409 OECD TG for solubility and dissolution rate testing is currently under development.<sup>22</sup> Whether this  
410 TG will actually address the traditional endpoint of solubility is not certain, as the focus is on the  
411 dissolution rate. According to Rasmussen et al.,<sup>33</sup> dissolution and dissolution rate are considered  
412 more relevant parameters than solubility for particulate materials. The ecotoxicological tests together  
413 with the bioaccumulation test can also be waived if the substance of concern is/has "unlikely/low  
414 potential to cross biological membranes". No exact methods are currently available to address this  
415 endpoint, however studies are reported in the scientific literature. Cellular uptake can be studied  
416 using e.g. flow-cytometry, microscopy, ICP-MS and TEM for cellular uptake<sup>61</sup> and in vitro assay(s)

417 used to study cytotoxicity and cytokine induction may also be relevant.<sup>64,65</sup> Lastly, biokinetics-  
418 oriented tests can be used to indicate whether a given NM is capable of cell penetration.<sup>66,67</sup> However,  
419 it is doubtful whether such approaches are enough to justify the potential activation of the waiving  
420 criteria.

421 If a substance is “readily biodegradable” a couple of the information requirements of the  
422 regulation can be waived. According to Baun et al.,<sup>91</sup> environmental biodegradability can be  
423 evaluated for carbonaceous materials via adaptation of the OECD biodegradation TGs series.<sup>68</sup> Here,  
424 a material is considered readily biodegradable if 60%/70% of the organic carbon in the material is  
425 converted to CO<sub>2</sub>/removed-within 28 days of testing. Again, the question arises on how to classify  
426 e.g. inorganic NMs with organic coatings. Biodegradability evaluations are only considered relevant  
427 for carbon-based NMs,<sup>91</sup> which points to a need to clarify which types of NMs this waiving criteria  
428 apply to.

429 In our view, the challenges identified here for justification of the activation of current waiving  
430 criteria in the revised REACH Annexes,<sup>4</sup> may be overcome by introducing more clearly defined  
431 methodological advices, but it is not clear whether such activities have been initiated.

432

433 *4.3 Focus on development of methods to determine adsorption/desorption, exposure scenarios and ability*  
434 *to cross biological membranes*

435 While Cefic has called for waiving of data generation until standards and test methods are  
436 defined for information requirements where they are currently unavailable,<sup>12</sup> ECHA has taken the  
437 stance that the lack of certain test methods should not be a hurdle in the registration process. In our  
438 analysis, we found that methods are currently lacking for assessing adsorption/desorption in aquatic  
439 environments, exposure scenarios and ability to cross biological membranes. Based on this, we tend  
440 to agree with the ECHA viewpoint. ECHA relies heavily on the OECD and ISO test  
441 guidelines/guidances/standards in their guidance to industry on registration and chemicals safety  
442 assessment of NMs. While some methods have been standardised, e.g. ISO 19590 standard for NP  
443 size measurement in aqueous media by single particle ICP-MS,<sup>50</sup> others are still under development,  
444 are lacking or are only applicable for some types of NMs.<sup>10</sup> While it is tempting to call for waiving of  
445 data generation until test methods are standardised, it is important to remember that the process of  
446 standardisation and TG development can be very timely and that the development of new methods  
447 often require targeted research effort over decades of times. Development of internationally accepted  
448 TGs and standards may be fostered by the increasing reporting demands on e.g. characterisation  
449 information of NMs such as the ones put in place by ECHA. This creates a clear societal need for the  
450 development of standards and motivates the registrants to develop and share their own methods and  
451 ideas on data that they have generated on a given endpoint. These should, however, be followed up  
452 by funding of research and development into the specific information requirements for which,  
453 methods, protocols and standards are currently lacking, namely for evaluation of  
454 adsorption/desorption behavior in aquatic environments, exposure scenarios and ability of NM to  
455 cross biological membranes.

456

457 *4.4. Regulatory adequacy of methods - reliability and relevance*

458 Besides need of methods to support the information requirements or to justify waiving, it is important  
459 to recognise that methods that are valid from a scientific perspective may not be seen as adequate  
460 from a regulatory perspective.<sup>92</sup> The term “regulatory adequacy” traditionally involves an evaluation  
461 of the relevance and reliability of a method or a study for the regulatory question at hand. Data of  
462 high regulatory relevance and reliability is therefore data that are well-suited for specific decision-  
463 making processes e.g. risk assessment and management,<sup>93,94</sup> whereas “scientific relevance and  
464 reliability” is more open-ended and often curiosity driven.<sup>95</sup>

465 In the field of nano-ecotoxicology the number of publications is rapidly increasing but the  
466 regulatory relevance and reliability of the reported findings has been questioned.<sup>96</sup> Today a couple of  
467 methods exist for assessing the regulatory adequacy of (eco)toxicological data, among them the  
468 NanoCRED<sup>97</sup> (available via the online tool SciRAP - Science in Risk Assessment and Policy -  
469 [www.scirap.org](http://www.scirap.org)). NanoCRED is an adjusted version of the “Criteria for Reporting and Evaluating

470 Ecotoxicity Data" (CRED) framework originally developed by Moermond et al.<sup>94</sup> as an alternative to  
471 the Klimisch method. The CRED framework is widely accepted for regulatory decision-making, e.g.  
472 recommended for evaluation of ecotoxicity data when setting environmental quality standards under  
473 the Water Framework Directive in the European Union.<sup>98</sup> We therefore suggest that more focus are  
474 put on the NanoCRED reporting recommendations. If registrants were required to carry out  
475 NanoCRED evaluations of their studies ECHA could publish these as part of the information on  
476 chemicals that they provide to enhance public access and transparency to the information. This will  
477 ensure that essential details are reported on when fulfilling the requirements of REACH Annexes but  
478 may also act as a guidance for registrants when performing studies.

## 479 **5. Conclusions and recommendations**

480 In this study, we assessed the degree of availability and suitability of methods needed to comply with  
481 the new technical data requirements to nanomaterials in the European Union's chemicals legislation  
482 for physico-chemical characterisation and environmental fate and effect assessment. In total, there  
483 are 20 additional, nano-specific information requirements for which methods are needed in order to  
484 register substances as nanofoms in REACH. For 15% of the information requirements, we found that  
485 internationally accepted TGs or standards are available. For 25% of the information requirements, we  
486 identified methods that are under development but which are expected to be developed into  
487 internationally accepted TGs or standards. Finally, we find that for 40 % of the information  
488 requirements, well-established methods are available through the scientific literature e.g. growth  
489 inhibition studies on aquatic plants and short-term toxicity testing on fish. Most of the relevant  
490 methods in the scientific literature are included in recent OECD GDs, ECHA guidance or ISO TRs  
491 and TS's. For four out of the 20 information requirements, we conclude that more research is needed  
492 to establish regulatory adequate methods. That is for methods related to quantification of  
493 adsorption/desorption, functional changes related to changes in e.g. hydrophobicity following  
494 exposure to aquatic environments and degradation. We also conclude that two of the 12 waiving  
495 criteria (17%) are supported by internationally accepted TGs or standards and for another 17% of the  
496 methods by internationally accepted TGs or standards under development. For 4 out of 12 waiving  
497 criteria, no methods were identified to support whether the criteria can be waived or not. We  
498 recommend that a targeted effort is made to develop methods, protocols and guidelines for  
499 information requirements and waiving criteria, which currently lack such information. To help fulfill  
500 the regulation information requirement on effects on terrestrial organisms, OECD GD 317 on Aquatic  
501 and Sediment Toxicological Testing of Nanomaterials<sup>32</sup> may be updated as noted by ECHA. We  
502 would, however, suggest that a GD on effects on terrestrial organisms is developed in parallel to the  
503 updates of OECD GD 317. It would further be helpful to clearly define and develop methodological  
504 advice on what it means for a NMs to be readily biodegradable, if the current information  
505 requirement are maintained. We also recommend that the development within the field of  
506 environmental exposure modelling of NMs should follow closely and be taken into account to help  
507 the registrants to justify "no exposure"/"low exposure" evaluations. We recommend that methods  
508 and/or guidance are developed to assess the waiving criteria of whether a NM is "likely to cross  
509 biological membranes". Certain information requirements and waiving criteria rely on partitioning  
510 methods, which are not deemed scientifically applicable to NMs. Instead of these, more relevant  
511 measures have been pointed to, e.g. dispersion stability<sup>14</sup> and dissolution rate<sup>17</sup>, and here NM-  
512 relevant guidance is available/under development. Finally, we recommend that increasing attention  
513 be put on reporting on regulatory reliability and relevance of the information that is submitted by  
514 the registrants. Specifically, this could include the adoption of NanoCRED reporting requirement for  
515 ecotoxicity studies and that ECHA makes these scores available as part of the information on NMs  
516 that they provide to enhance public access to information.

### 517 **Conflicts of interest**

518 There are no conflicts to declare.

### 520 **Acknowledgments**

522 The financial support from the research program Mistra Environmental Nanosafety Phase II, funded by the  
523 Swedish Foundation for Strategic Environmental Research (Mistra), and the PATROLS – Advanced Tools for  
524 NanoSafety Testing under Horizon 2020 research and innovation programme, is greatly acknowledged. Jonas  
525 Hedberg, from KTH Royal Institute of Technology Department Chemistry, is acknowledged for his help with  
526 identifying methods for information requirements and waiving criteria on physico-chemical properties and  
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