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

Maria Bille Nielsen[1]*, Anders Baun[1], Aiga Mackevica[1], Amalie Thit[2], Inger Odnevall Wallinder[3], Julián Alberto Gallego[4], Lauge Peter Westergaard Clausen[1], Jenny Rissler[5], Lars Skjolding[1], Alejandra Castro Nilsson[6], Tommy Cedervall[7], Steffen Foss Hansen[1]

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[1] and by engineering the material characteristics at the nanoscale such as size, shape and surface coating to fit a specific purpose.[2] This can change properties such as reactivity, surface composition, and dispersion stability, causing the nanomaterials to behave differently from their macroscale material counterparts.[1-3]

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

[1] Department of Environmental Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

* Corresponding author: mabini@env.dtu.dk

[2] Department of Science and Environment, Roskilde University, 4000 Roskilde, Denmark

[3] KTH Royal Institute of Technology, Department Chemistry, Division Surface and Corrosion Science, 114 28 Stockholm, Sweden

[4] Department of Marine Sciences, University of Gothenburg, Kristineberg marine research station, 451 78 Fiskebäckskil, Sweden

[5] Department of Design Sciences, Lund University, Box 118, 221 00 Lund, Sweden & Department of Material and Surface Design, RISE Research Institutes of Sweden, SE-223 60 Lund, Sweden

[6] SOLVE Research and Consultancy AB, Medicon village, SE-22381 Lund, Sweden

[7] Department of Biochemistry and Structural Biology, Lund University, Box 117, 221 00, Lund, Sweden

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

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

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

Application of conventional methods to test these parameters is often challenged by the specific properties of NMs and their non-equilibrium system-dependent behaviours. This has imposed the need for new or modified methods.[10]

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

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

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

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

2. Materials and Methods

2.1 Literature review

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

2.2 Categorisation of methods

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

3. Results

For all the information requirements listed in the REACH Annexes,[4] we gathered information about the availablity of metods, protocols and guidelines from OECD, ISO and ECHA and their respective databases and catalogues as well as the scientific literature. Table 1 provides an example of information compiled for the first information requirement, namely 7.7 Solubility (and dissolution rate). A full overview of all information collected for each information requirement is given in Electronic Supplementary Material 1 and 2.

Table 1. Example of information collected on the availablity of metods, protocols and guidelines for the information requirement 7.7 Solubility (and dissolution rate) and its waiving criteria and the highest-ranked category of identified methods needed to acquire/fulfill the information requirement and its waiving criteria set by the European chemicals legislation REACH for nanomaterials (NMs). Details for the full overview is given in Electronic Supplementary Material 1. TGS: Internationally accepted test guideline or standard; TGSUD: Internationally accepted test guideline under development; SCI: Established as standard method in scientific literature; O: Other method 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: TR: technical report; GD: guidance document; Organisation for Economic Co-operation and Development (OECD); ISO: International Organization for Standardization; ECHA: the European Chemicals Agency. WNT: Working Group of the National Coordinators for the Test Guidelines Programme.

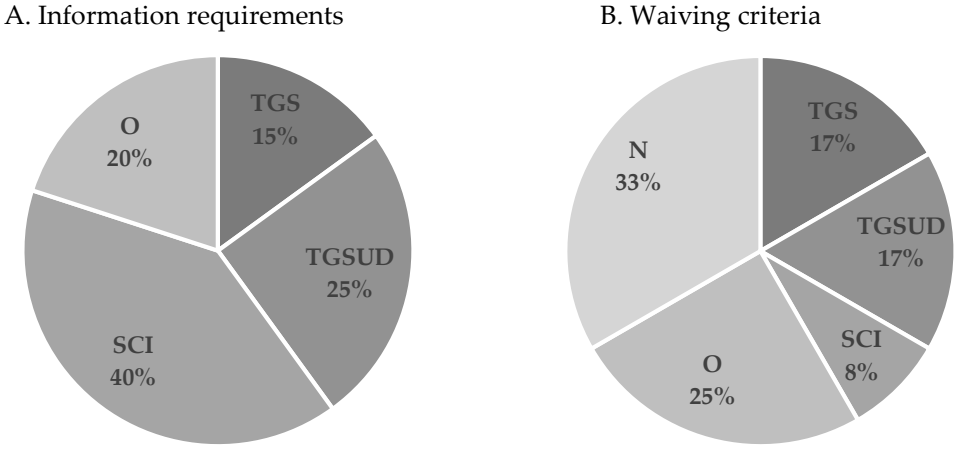
Information requirement	Waiving criteria	Methods, protocols and guidance	Highest method category
7.7 Solubility (and dissolution rate)		• OECD WNT Project 3.10: New TG on dissolution rate of NMs in aquatic environment ongoing.[22] • OECD WNT Project 1.5: GD on determination of solubility and dissolution rate of NMs in water and relevant synthetic biological media ongoing for new guidance document/test guideline.[22] • GD 318 for the testing of dissolution and dispersion stability of NMs, and the use of the data for further environmental testing and assessment.[23] The GD lists the following methods: 1. Batch test using Centrifugal ultrafiltration (not recommended ultracentrifugation or dialysis) 2. Dynamic testing of dissolution rates based on Koltermann-Jülly et al.[24] • Partially covered in ECHA Guidance.[13,14] • OECD TG 105[25]: not adapted to NMs specifically • ISO/TR 19057[26]: adapted to NMs • OECD GD 29 on Transformation/ Dissolution of Metals and Metal Compounds in Aqueous Media[27] is applicable for some NMs. E.g., Wasmuth et al.[28] applied it successfully to silver NMs. • Multiparameter matrix methods may be used to study dissolution and solubility. E.g., Xiao et al.[29] performed a multifactorial design for the water chemistry (based on OECD-TG-318 matrix of parameters: natural organic matter, ionic strength and pH). • Study of dissolution of metal NMs environmental (freshwater) conditions.[30,31]	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.[22] • **	TGSUD

** 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,[32] 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[6] 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

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

Table 2. The 20 additional, nano-specific information requirements for registration of nanomaterials (NMs) in the European chemicals legiaslation (REACH)[4] and categories of identified highest-ranked method needed to acquire/fulfill the information requirements. TGS: Internationally accepted test guideline or standard; TGSUD: Internationally accepted test guideline or standard under development; SCI: Established as standard method in scientific literature; O: Other method 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: TR: technical report; TS: technical report; GD: guidance document; Organisation for Economic Co-operation and Development (OECD); ISO: International Organization for Standardization; ECHA: the European Chemicals Agency; WNT: Working Group of the National Coordinators for the Test Guidelines 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[22]; OECD WNT Project 1.5: GD under development[22]; OECD GD 318[23]; ECHA Guidance R7.1a and R7.1b appendices** [13]; OECD GD 29[27]: applicable for some NMs; Multiparameter matrix methods[e.g. 29,35]; Dissolution study of metal NMs environmental (freshwater) conditions.[30,31]
7.8 Partition coefficient n-octanol/ water *(VII)*	O	ECHA Guidance**.[13,14]
7.14bis Dustiness *(VII)*	TGSUD	OECD WNT Project 1.8: TG under development[22]; Rotating drum method, continuous drop method and small rotating drum method,[33,36] for which British standards exist: EN 15051-1,[37] 15051-2[38] and EN 15051-3.[39]
9.1.1 Short-term toxicity testing on invertebrates *(VII)*	SCI*	ISO/TS 20787:2017[40]; OECD GD 317.[32]
9.1.2 Growth inhibition study aquatic plants *(VII)*	SCI*	OECD GD 317.[32]
7.14ter Further information on physico-chemical properties *(VIII)*	TGS	OECD decision tree[14]; OECD framework on physical chemical characterisation[14]; OECD WNT Project 1.7: TG under development[22]; ISO/TR 11360:2010.[14,41]
9.1.3 Short-term toxicity testing on fish *(VIII)*	SCI*	OECD GD 317.[32]
9.1.4 Activated sludge respiration inhibition testing *(VIII)*	SCI*	OECD GD 317.[32]
9.2 Degradation *(VIII)*	O	OECD WNT 3.16 Project: GD under development.[22] Qualitative assessment may be possible in the meantime.[14]
9.2.2.1 Hydrolysis as function of pH *(VIII)*	TGS	OECD TG 318.[34]
9.3.1 Adsorption/desorption screening *(VIII)*	O*	ECHA Guidance 2017** [13,14]; Dye staining to measure hydrophobicity of NMs in aquatic environments.[42]

9.2.1.2 Simulation testing on ultimate degradation in surface water *(IX)*	TGS	OECD TG 318.[34]
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[e.g. 43-45]; In vivo and in vitro methods for acellular dissolution in physiological fluids[e.g. 36]; OECD WNT Project 3.12: GD under development.[22]
9.3.3 Further information on adsorption desorption depending on the results of the study required in Annex VIII *(IX)*	O	ECHA Guidance** [13,14]; Dye staining to measure hydrophobicity of NMs in aquatic environments.[42]
9.4 Effects on terrestrial organisms *(IX)*	SCI	OECD GD 317[32] may be updated for terrestrial organisms[14]; Equilibrium partitioning method may be applied, if scientifically justified[4]; Proposal on OECD TG 312[46] as alternative to equilibrium partitioning method[47,48,13]; Draft OECD GD on testing nanomaterial behaviour in soils using OECD TG 312 under development[14]; OECD TG 303A[49] 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[22]; ISO/TS 19590:2017[50]; ISO 22412:2017[51] applies, with certain restrictions[33]; ISO/TS 21362:2018[52]; Imaging methods, e.g. electron microscopy[53]; Nanoparticle Tracking Analysis (NTA).[54]
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,[6] including analytical techniques (e.g. IR, NMR, TGA, ICP-MS, XRF, XPS, EDX, GC-MS, MALDI-TOF, etc.), NANoREG[55] and ISO TR 14187:2011.[56] Revised version: ISO/TR 14187:2020.[57]
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[22]; Protocols from research projects and/or standard methods and/or scientific literature available for determination of crystallinity/assembly structure[14]; Electron microscopy (EM) for determination of shape.[33]
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[22]; ISO/TR 14187:2011[56,57] provides some input to relevant information; ISO 9277:2010[58] applies, with certain restrictions; Determination of the specific surface area of solids by gas adsorption — BET method, with restrictions.[33]
5.2.3 Characterisation the particle aggregation and the agglomeration *(I)*	SCI	Certain imaging methods, such as electron microscopy[53,59]; Electron microscopy image and transmission electron topography[60,61]; Draft OECD TG on Agglomeration Behaviour of

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

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

262 ** Information requirement only partially covered by ECHA Guidance.[11]

263

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.[4] 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.[e.g. 43-45]

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.

Table 3. Waiving criteria for information requirements for registration of nanomaterials (NMs) in the European chemicals legislation (REACH) and category of identified highest-ranked method needed to assess if waiving criteria are triggered. TGS: Internationally accpted test guideline or standard; TGSUD: Internationally accepted test guideline or standard under development; SCI: Established as standard method in scientific literature; O: Other method and/or more research needed; N: No method identified. The method categories are ranked highest to lowest: TGSS, TGUD, SCI, O and N. Other abbreviations: TR: technical report; TS: technical report; GD: guidance document; Organisation for Economic Co-operation and Development (OECD); ISO: International Organization for 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.[22]
(7.8) The substance is inorganic	O	*No method needed.*
(7.14bis) Exposure to granular form of the substance during its life-cycle can be excluded	N	*No method identified.*
(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[22]; OECD WNT Project 1.5: GD under development.[22]
(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[61]; In vitro assay(s) for cytotoxicity and cytokine induction[64,65]; Biokinetics-oriented tests for capability of cell penetration.[66,67]
(9.2.2.1; 9.2.1.2; 9.1.4) The substance is readily biodegradable	TGS	OECD TG 301A-F.[68]
(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	*No method identified.*
(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*** [13,14]; Dye staining to measure hydrophobicity.[42]
(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[e.g. 43-45]; In vivo and in vitro methods for acellular dissolution in physiological fluids[e.g. 36]; OECD WNT Project 3.12: GD under development.[22]

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

* Hydrolytically NMs are in this context considered unstable or readily oxidisable or rapidly dissolving (half-life less than 12h); ** For nanoforms, the study may not be waived on the basis of high insolubility in water alone[4]; *** Information requirement only partially covered by ECHA Guidance.[11]

Table 4. Additional information requirements and the criteria for triggering the additional requirements for registration of nanoforms in REACH and category of identified highest-ranked methods needed to acquire/fulfill information requirements and associated criteria. TGS: Internationally accepted test guideline or standard; TGSUD: Internationally accepted test guideline or standard under development; SCI: Established as standard method in scientific literature; O: Other method and/or more research needed; N: No method identified. For further details, see Electronic Supplementary Material B. The method categories are ranked highest to lowest: TGS, TGSUD, SCI, O and N. Other abbreviations: TR: technical report; TS: technical report; GD: guidance document; Organisation for Economic Co-operation and Development (OECD); ISO: International Organization for Standardization; ECHA: the European Chemicals Agency; WNT: Working Group of 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[22]; OECD GD 318.[23]
	(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[34] 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[22]; OECD WNT Project 1.5: GD under development[22]; OECD GD 318.[22]
	(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.[69]
(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, article stability/mobility in solution etc. Surface speciation and chemical speciation predication in solution is essential to asses.[31]
(8.8.1) For nanoforms without high dissolution rate in biological media		TGSUD	OECD WNT Project 3.10: TG under development[22]; OECD WNT Project 1.5: GD under development[22]; OECD GD 318[23]; ECHA Guidance R7.1a and R7.1b appendices* [13]; OECD GD 29[27]: applicable for some NMs; Multiparameter matrix methods[e.g. 29,35]; Dissolution study of metal NMs environmental (freshwater) conditions.[30,31]
	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[22]; OECD WNT Project 1.5: GD under development[22]; OECD GD 318[23].
	(9.1.3) The long-term aquatic toxicity study on fish (Annex IX, Section 9.1.6) shall be considered	TGS	OECD TG 204[70].
(9.2) Nanoforms that are not soluble, nor have high dissolution rate		TGSUD	OECD WNT Project 3.10: TG under development[22]; OECD WNT Project 1.5: GD under development[22]; OECD GD 318[23].
	(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.[e.g. 36]; Redox potential measurements.[e.g. 71,72]
	(9.2) Chemical transformation (e.g. oxidation, reduction)	SCI	Combining TEM with energy-dispersive X-ray spectroscopy (EDX) for elemental composition of the nanoparticles[61,73]; Attenuated Total Reflection Fourier transform infrared Spectroscopy (ATR-FTIR)[74;] Atomic force microscopy (AFM) – changes in particle morphology and size[e.g. 75]; Zeta potential measurement[76] and reporting[77]; X-ray Absorption Spectroscopy.[78]
	(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[74] and EXAFS[79]; OECD TG 312[46] is applicable for NMs[13,47,48,]; Retention determination of silver and

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

313 ***** *Information requirement only partially covered by ECHA Guidance.*[11]

4. Discussion

4.1. Information requirements

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

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

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

Attachment and Removal from Wastewater[49] can furthermore be used as an alternative approach to predict sorption of NMs.

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

4.2. Waiving criteria

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

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

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

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

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

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

If a substance is "readily biodegradable" a couple of the information requirements of the regulation can be waived. According to Baun et al.,[91] environmental biodegradability can be evaluated for carbonaceous materials via adaptation of the OECD biodegradation TGs series.[68] Here, a material is considered readily biodegradable if 60%/70% of the organic carbon in the material is converted to CO_2/removed-within 28 days of testing. Again, the question arises on how to classify e.g. inorganic NMs with organic coatings. Biodegradability evaluations are only considered relevant for carbon-based NMs,[91] which points to a need to clarify which types of NMs this waiving criteria apply to.

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

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

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

4.4. Regulatory adequacy of methods - reliability and relevance

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

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

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

5. Conclusions and recommendations

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

Conflicts of interest

There are no conflicts to declare.

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