Gov4Nano
meeting the needs of nanotechnology

Deliverable Report

Deliverable D 2.1.1/D2.1	Draft Guidance document on nanomaterial characterization guidelines and their application in regulation
Due date of deliverable	*2019/12/31*
Approved by MC	*2020/03/05*
Actual submission date	*2020/01/31*
File name	D2.1_G4N_ Draft Guidance document on nanomaterial characterization guidelines and their application in regulation_V1.docx

Author(s) and beneficiary acronym:	Lead authors: Volker Bachmann – BauA Elisabeth Heunisch – BAuA Contributions from: Eric Bleeker – RIVM David Carlander – NIA Melanie Müller – BAuA Thomas Kuhlbusch - BAuA
Work package/task:	WP2 / Task 2.1
Document status:	draft / final
Confidentiality:	confidential / restricted / public
Key words:	

DOCUMENT HISTORY

Version	***Date***	***Reason of change***
1	2020/01/31	
2		
3		
4		

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement 814401

Lead beneficiary for this deliverable: Federal Institute for Occupational Safety and Health, BAuA, 03

Owner(s) of this document	
Owner of the content	BAuA (3)
Co-Owner 1	RIVM (1)
Co-Owner 2	NIA AISBL (7)

Dissemination Level:			
PU	Defined in the DoA	Public	PU PP RE CO *Underline only one of the options*
PP		Restricted to other programme participants (including the Commission Services)	
RE		Restricted to a group specified by the consortium (including the Commission Services)	
CO		Confidential, only for partners of the consortium (including the Commission Services)	

1.	Gov4Nano Advisory Board	Yes
2.	NMBP-13 projects collaboration core groups	Yes
3.		Yes/No
4.		Yes/No
5.		Yes/No
6.		Yes/No
7.		Yes/No

Table of Content

1 Summary

2 Description of task

The original task of this deliverable was to development an overarching guiding document on standards and test methods being fit for use for nanomaterials in regulation e.g. on how results and information from these tests can be combined to facilitate nanomaterial exposure, hazard and risk assessment (in close collaboration with WP4). The document to be developed is foreseen to give an overview on the fitness of test guidelines for nanomaterials, how these guidelines are interlinked for e.g. transformation, exposure, hazard or risk assessments, and safer-by-design as well as indicating which further evaluation and development steps are needed or advantageous to better assess nanomaterials. Some part of this task is covered in parallel by the Project NANORIGO D1.2 Overview on guidelines for nanotechnology: material, exposure, hazard, risk.

Due to this successful cooperation, a split of work was agreed and further, more detailed analyses, descriptions of standardization and harmonization bodies and guidance on when to choose which type of Standard/Guideline was and will be worked on.

In this document, we summarise the status and outline future activities, possibly done in cooperation with NANORIGO.

3 Description of work & main achievements

3.1 Background of the task

Regulation of chemicals agents, e.g. by the chemical agents directive 98/24/EC, if standards and harmonised test method for evaluation of the safety are available. With the development of nanomaterials, the discussion on their possible risks and a need for testing and evaluation identified. This was identified by the European Union and by Commissions Regulation 2018/1881 amending the REACH Annexes a requirement for providing information on nanomaterials on the EU market was agreed. This regulation came into effect by January 2020.

Considering this background the combined work in NanoRigo and Gov4Nano on listing and evaluating the available test methods and standards for nanomaterials is important. Additionally, giving guidance on when which standardisation or harmonisation pathway should be used (national test methods, ECHA Guidances, ISO/CEN standards, OECD Test Guidelines) are an important prerequisite to facilitate a practicable as well as an ecological-economical balanced regulatory approach.

3.2 Description of the work carried out

3.2.1 Overview on Standardisation Bodies and Organisations

'ISO was founded with the idea of answering a fundamental question: "what's the best way of doing this?"' This statement from the ISO webpage is the motive behind any kind of standardization process. In this segment, the four most important organizations or legal bodies to set standards are briefly described.

3.2.1.1 OECD Test Guidelines and Guidance Documents

3.2.1.1.1 Aim and Purpose

OECD Test Guidelines (TGs) for the testing of chemicals are internationally agreed documents and describe procedures on how to assess the safety of chemicals. In total, they comprise the most relevant end points in safety assessment and are used by governments, industry and independent laboratories. The prime use is 'in regulatory safety testing and subsequent chemical notification and registration.' [1] The member countries to the OECD update and extend the Test Guidelines regularly to stay up to date with the latest developments in science and technology.

All OECD Member Countries and Countries adherent are bound to the system of "Mutual Acceptance of Data – MAD". By this, the Test Guidelines are standards in all member countries and avoid contradicting national testing, which would become trade barriers. Furthermore, this system is to avoid the double testing of substances within the OECD member countries in order to save time and money and to reduce the number of animal testing. If the safety assessment is conducted according to an OECD Test Guideline and the Principles of Good Laboratory Practice the results have to be accepted in all member countries.

3.2.1.1.2 Participating Persons and Institutions

The OECD, the Organization for Economic Cooperation and Development, is an organization of currently 36 member countries, mainly industry nations but also strong emerging economies. Furthermore, there is cooperation with emerging economies like China, India or Brazil and a close collaboration the European Commission. Argentina, Brazil, India, Malaysia, Singapore and South Africa are countries with full adherence to MAD.

Acting persons on the different levels of OECD have to be officially nominated and are representatives of their individual country. Additionally other stakeholders like industry, standardization bodies like ISO and NGOs take part in the discussions on the different working levels, e.g. towards new OECD Test Guidelines towards chemicals safety testing.

3.2.1.1.3 Decision and Adaptation Process

The work on OECD Guidelines is conducted in the Working Group of National Coordinators of the TGs programme (WNT) under the Environment Directorate of OECD. In order to adapt or newly develop an OECD Guideline, one (or more) member country or stakeholder has to come forward to the WNT with a proposal to do so. Upon approval the lead country/stakeholder develops a draft TG using sound scientific data and consulting with experts in the field. Once the draft TG is submitted to the WNT it undergoes a commenting process by member country and stakeholder experts which are nominated by their country/organization according to their field of expertise. The end of the commenting process is reached once the final draft TG finds approval in the WNT. Final step prior to publication and coming into effect is the adoption of the draft TG by the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology(JM). The finally published OECD Guideline can be accessed worldwide free of charge.

3.2.1.1.4 Legal Relevance

Through the Mutual Acceptance of Data results obtained by following an OECD Test Guideline have to be accepted among all countries bound to MAD. In the European Union the chemicals regulation REACH often refers to OECD Test Guidelines in its own guidances, see below, to help registrants fulfill their information requirements on the safe use of substances. Furthermore, the Test Method Regulation is based largely on OECD TGs, see below. Other member countries as well refer to the OECD Test Guidelines in their national chemicals regulation.

The OECD Test Guidelines by itself do not have the status of an international applicable law, but lawmakers in the OECD member countries and possibly beyond refer to those Guidelines once drafting laws regarding the chemicals safety assessment.

3.2.1.2 ISO/CEN

3.2.1.2.1 Aim and Purpose

ISO is a non-governmental organization in which 164 national standardization bodies are joined together. It was founded in 1946 with the intention 'to facilitate the international coordination and unification of industrial standards' [ISO About us]. Issued standards are categorized in 40

different fields and span the broad field from organizational issues over metrology and testing to specific industry branches.

Aim and purpose of the development of ISO and its regional and national counterparts, e.g. CEN as European Standardization Organization and DIN as the German Standardization Organization, respectively, is the documentation of best practices for qualitative, safe and efficient production and use of goods, services and organizational processes. According to the ISO webpage, the agreement on international standards is seen as an instrument of facilitating international trade.

3.2.1.2.2 Participating Persons and Institutions

Members to ISO are the national standardization organizations, like DIN in Germany, JISC in Japan or ANSI in the USA. The acting persons in the currently 781 technical committees and subcommittees are present on behalf of their national organization, which has national mirror committees. Becoming a member to the national committees is possible for all interested stakeholders like industry, consumers, trade, academia, public authorities, or research and testing institutes. For a membership, companies and associations have to pay fees dependent on the size of the company/association and on the amount of technical committees they are involved in.

3.2.1.2.3 Decision and Adaptation Process

Drafting and agreement of an ISO standard is on consensus of all participating parties in a particular standardization project. Delegates will represent their national interests and will see to it that the drafted ISO standards are not in contradiction to national standards. Further reading on how National, European and International Standards are developed can be found on e.g. the DIN webpage [DIN About Standards].

3.2.1.2.4 Legal Relevance

ISO standards or regional and national standards are by themselves no legal document. Nevertheless, regulators can choose to reference ISO and other standards in legally binding documents, just like mentioned above for OECD Test Guidelines.

As they are internationally agreed upon ISO and other standards are used as terms of reference in documents like OECD Test Guidelines or ECHA Guidance Documents.

3.2.1.3 ECHA Guidance

3.2.1.3.1 Aim and Purpose

The European Chemicals Agency (ECHA) issues Guidance Documents to help implementing the European Chemicals Regulation REACH (Registration, Evaluation, Authorisation and restriction of CHemicals). Those Guidance Documents contain descriptions of good practices on how to fulfil the obligations. It also offers Guidance Documents for the biocide regulation and CLP, the regulation for classification, labeling and packaging of products.

Guidance on REACH is structured according to the legal text and can be found in completeness on the ECHA website [ECHA Guidance on REACH].

3.2.1.3.2 Participating Persons and Institutions

Guidance documents and other support products like factsheets, practical guides and others are developed with participants from industry, Member States and NGOs. The work is organized in expert groups, like the Nanomaterials Expert Group (NMEG) where the Member States nominate 1-2 experts in the field and further experts are nominated by the accredited stakeholder organisations (ASOs). Within the ASOs mainly industry associations are listed, but also environmental and animal welfare NGOs, consumer and academic associations and trade unions.

3.2.1.3.3 Decision and Adaptation Process

The adaptation of ECHA Guidance or the new draft of ECHA Guidance is driven by the scientific and regulatory development in the field. The adaptations towards nanomaterials were triggered

by the rapid developments in the scientific community and by the discussions on the development of nano specific amendments to the REACH Annexes, as an example. This led to a draft of three new appendixes under Part B of the Guidance on Information Requirements and Chemical Safety Assessment, published in May 2017, namely to the chapters R.7a-c on Endpoint specific guidance [Appendix R7-1 to Chapter R7a (pdf)], [Appendix R7-1 to Chapter R7b (pdf)], [Appendix R7-2 to Chapter R7c (pdf)]. More recently appendixes for nanoforms were published on the Guidance on substance identification [Appendix Nanoforms Registration and Substance ID] and to Chapter R.6 under Part B of the Guidance on Information Requirements and Chemical Safety Assessment QSARs and grouping of chemicals [Appendix R.6-1 to Chapter R6 (pdf)], both in December 2019.

New Guidance documents or adaptation are decided upon by consensus in the drafting expert group and undergoes rounds commenting and consensus in relevant ECHA committees. For the above mentioned appendixes on nanoforms the Committee for Risk Assessment (RAC) and the Member State Committee (MSC) were chosen. Afterwards, on the level of the European Commission, the expert group of the Competent Authorities for Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) and Classification, Labelling and Packaging (CLP) (CARACAL) will comment if necessary and upon consensus with this expert group the new or adapted Guidance is published on the ECHA website.

3.2.1.3.4 Legal Relevance

As for OECD and ISO Guidance documents, the ECHA Guidance documents are not legally binding. Registrants can choose to follow the documents and therefore best practices to comply with the information requirements, but are free to divert from it. Once they choose other methods a scientific explanation has to be given on the information requirements are still met.

3.2.1.4 Test Method Regulation (EC) No 440/2008

3.2.1.4.1 Aim and Purpose

REACH regulation (EC) No. 1907/2006 states that it is necessary to have harmonized test methods to collect information on the inherent substance properties. This information has to be provided in a dossier to the European Chemicals Agency (ECHA) containing all information requirements according to the REACH regulation. In appendix V to regulation 67/548/EEC methods to determine physical-chemical properties, toxicity and ecotoxicity of substances and formulations. The Test Method Regulation includes the methods from 67/548/EEC and replaced it in 2008. In 2017, regulation (EU) 2017/735 was issued to amend/update (EC) No. 440/2008 due to technical advancements.

3.2.1.4.2 Participating Persons and Institutions

The initiative for the Test Method Regulation is with the European Commission. The initial regulation and its update from 2017 are drafted and refined by experts from the EC and Member States and undergoes public consultation where stakeholders from e.g. industry, consumer and animal welfare NGOs, academia or trade unions are most likely to comment on the draft and suggest changes to the draft.

3.2.1.4.3 Decision and Adaptation Process

The Test Method Regulation is a piece of European legislation. Therefore, the European Commission is responsible for drafting the text followed by a consultation process and negotiation phase. The European Parliament is the legislative branch in the EU who has to pass the regulation by majority vote.

3.2.1.4.4 Legal Relevance

The methods in this regulation are legally binding, but the recital states that alternate methods can be used if they comply the article 13 (3) of the REACH regulation.

About half of the methods contained in this regulation are reprints of or based on the respective OECD Test Guidelines on the respective endpoints, namely 46 of the 80 endpoints.

3.2.2 Existing Standards

In the Annex of this document one table summarizes the nano-specific endpoints in REACH Annexes VI-X and lists existing standards. Input to this collection has been provided by JRC (Gov4Nano D6.9) and by NANORIGO D1.2.

3.2.3 Gaps in Standards

Specific steps to be conducted within or suggested for the next period are

- Gap analyses by comparison of the List of standards/test methods with the REACH Annex requirements as well as recommendation by the ECHA NanoMaterial ExpertGroup,
- Analyses of other European regulations related to nanomaterials and their testing/evaluating requirements,
- A meeting of the researchers involved in D6.9 of Gov4nano, D1.2 NanoRigo and D2.1 Gov4Nano to systematically discuss end points and status of evaluation/testing possibilities,
- Summary of the status of nanomaterial testing for European regulation, focus REACH, to be written and made publicly available.

3.3 Results

This is a collection of information, which will be updated according to the technical and scientific development during the course of this project. A first overview of available information including limited analyses was achieved based on information from D 6.9 of Gov4nano and D1.2 NANORIGO. Additionally the main international standardisation and harmonisation bodies are described and first discussions on prioritising or choosing which way to follow for safety evaluation of nanomaterials have been conducted.

3.4 Evaluation and conclusions

This deliverable is the draft version of D2.1.3/D2.3 "Final Guidance document on nanomaterial characterization guidelines and their application in regulation" which is due in M48 of the project. It collects the current status of guidelines, regardless whether OECD, ECHA, or ISO standards, on the four group of information requirements substance identification, physical-chemical characterization, toxicity and eco-toxicity.

In the course of the project it will be continued to develop this document towards comprehensive collection on all relevant nano-specific endpoints, a "one stop shop", that will allow the reader for quick and easy access to all available and relevant and as standards considered documents. Eventually a database with web-tool is envisaged for an even easier access to those documents.

3.5 Data management – only for a limited number of tasks relevant

Does not apply.

4 Deviations from the work plan

Timely delay of one month due to parallel ongoing activities. The split of work was newly discussed between D6.9 Gov4nano and D 1.2 NANORIGO. Based on this discussion the focus of this D2.1 was extended to also include recommendations when to use or develop which standard/Guideline. The detailed gap analyses is still a main action to be part of the update of this Deliverable (D2.3).

5 Performance of the partners

Good cooperation between the lead beneficiary BAuA and the contributing partners RIVM and NIA.

6 References / Selected sources of information

Webpage of ISO

Webpage of OECD

Webpage of DIN

Webpage of ECHA

Gov4Nano Deliverable D6.9 "Risk governance standards baseline report"

NANORIGO Deliverable D1.2 "Overview on guidelines for nanotechnology: material, exposure, hazard, risk"

Annexes

Information in the tables of the annexes were provided by the lead author of Gov4Nano deliverable D6.9 and NANORIGO deliverable D1.2.

Table 1: REACH Endpoints Identity of NMs

Requirement/endpoint in REACH (2018)	REACH Annex	OECD TG/GD/report	Standards ISO/CEN	Peer reviewed	project reports
2.4.2: Number based particle size distribution	Annex VI	• New TG on particle size and size distribution of Manufactured Nanomaterials (project 1.4 - DE)	• ISO 13318-2:2007, Determination of particle size distribution by centrifugal liquid sedimentation methods — Part 2: Photocentrifuge method • ISO 17867:2015, Particle size analysis — Small-angle X-ray scattering • ISO 19430:2016, Particle size analysis — Particle tracking analysis (PTA) method • ISO 22412:2017, Particle size analysis — Dynamic light scattering (DLS) • CEN ISO/TS 19590:2019, Nanotechnologies — Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry • ISO/TS 21362:2018, Nanotechnologies — Analysis of nano-objects using asymmetrical-flow and centrifugal fieldflow fractionation		

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement 814401

			• Under development: • ISO/DIS 19749, Nanotechnologies — Measurements of particle size and shape distributions by scanning electron microscopy • ISO/DIS 21363, Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy		
2.4.3: Surface functionalisation	Annex VI	• New GD on Identification and quantification of the surface chemistry and coatings on nano- and microscale materials (project 1.6 - DK, DE) under development from 2019.	• ISO 13099-1:2012, Colloidal systems — Methods for zeta-potential determination — Part 1: Electroacoustic and electrokinetic phenomena • ISO/TR 19997:2018, Guidelines for good practices in zeta-potential measurement • ISO/TS 14101:2012, Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method • ISO 20579-4:2018, Surface chemical analysis — Guidelines to sample handling, preparation and mounting— Part 4: Reporting information related to the history, preparation, handling and mounting of nano-objects prior to surface analysis		
2.4.4: Shape, aspect ratio and other morphological chartacterisation	Annex VI	• Advice from TG on particle size and size distribution of Manufactured Nanomaterials (length and aspect ratio for fibres (elongated particles). 2D nanomaterials not covered by the TG. (Project 1.4 - DE)	• ISO/TR 19733:2019, Nanotechnologies — Matrix of properties and measurement techniques for graphene and related two dimensional (2D) materials		
2.4.5: Surface area	Annex VI	• New TG on Determination of the (Volume)Specific Surface Area of Manufactured Nanomaterials (Project 1.3 - EU/JRC) - finalisation expected by 2021	• ISO 9277:2010, Determination of the specific surface area of solids by gas adsorption — BET method		

			• ISO 12154:2014, Determination of density by volumetric displacement — Skeleton density by gas pycnometry • ISO 18747-1:2018 Determination of particle density by sedimentation methods — Part 1: Isopycnic interpolation approach • ISO 18747-2:2019, Determination of particle density by sedimentation methods — Part 2: Multi-velocity approach		

Table 2: REACH Endpoints physical-chemical characterization of NMs

Requirement/endpoint in REACH (2018)	REACH Annex	OECD TG/GD/report	Standards ISO/CEN	Peer reviewed	project reports
7.7 Water solubility	Annex VII	• New GD on Determination of solubility and dissolution rate of nanomaterials in water and relevant synthetic biological media (Project 1.5 - DK, DE) • New TG on dissolution of metal nanomaterials in aquatic media (Project 3.10 - US) • New GD on agglomeration and dissolution behaviour in aquatic media, expected to be available presumably in 2020 (Project 3.9 - DE)	• ISO/TR 13097:2013 , Guidelines for the characterization of dispersion stability		
7.8 Partition Coefficient n-Octanol/water	Annex VII	• TG 318: Dispersion stability (not offering advice on differentiation between dissolution and dispersion) • Guidance on dissolution rate and dispersion stability is expected presumably in 2020 (Project 3.9 - DE)			
7.14 Granulometry Particle Size Distribution	Annex VII	•			
7.14bis Dustiness	Annex VII	• New TG on Determination of the Dustiness of Manufactured Nanomaterials (Project 1.8 - FR)	• ISO/TS 12025:2012, Nanomaterials — Quantification of nano-object release from powders by generation of aerosols • ISO/TS 21361:2019, Nanotechnologies — Method to quantify air concentrations of carbon black and amorphous silica in the nanoparticle size range in a mixed dust manufacturing environment • EN 17058:2018, Workplace exposure - Assessment of exposure by inhalation of nano-objects and their aggregates and agglomerates • EN 17199-1:2019, Workplace exposure - Measurement of dustiness of bulk		

			materials that contain or release respirable NOAA and other respirable particles - Part 1: Requirements and choice of test methods • EN 17199-2:2019 WI=00137058) Workplace exposure - Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 2: Rotating drum method 2019-03-27 • EN 17199-3:2019, Workplace exposure - Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 3: Continuous dropmethod • EN 17199-4:2019, Workplace exposure - Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 4: Small rotating drum method • EN 17199-5:2019, Workplace exposure - Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 5: Vortex shaker method • EN 16897:2017, Workplace exposure - Characterization of ultrafine aerosols/nanoaerosols - Determination of number concentration using condensation particle counters • EN 16966:2018, Workplace exposure - Measurement of exposure by inhalation of nano-objects and their aggregates and agglomerates - Metrics to be used such as number concentration, surface area concentration and mass concentration		

			• EN ISO 28439:2011, Workplace atmospheres - Characterization of ultrafine aerosols/nanoaerosols - Determination of the size distribution		
7.14ter Further information on physicochemical properties	Annex VIII	In addition, WNT Project 1.7, an OECD Test Guideline (Malta Initiative) a TG on hydrophobicity is under development, which can be used to provide further information on physical chemical properties.	• ISO/TR 11360:2010 describes a classifying system, termed a “nano-tree”, upon whose basis wide ranges of nanomaterials can be categorized, including nano-objects, nanostructures and nanocomposites of various dimensionality of different physical, chemical, magnetic and biological properties. • ISO/TR 13014:2012, Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment ISO/TR 18196:2016, Nanotechnologies — Measurement technique matrix for the characterization of nano-objects		

Table 3: REACH Endpoints human toxicity of NMs

Requirement/endpoint in REACH (2018)	REACH Annex	OECD TG/GD/report	Standard ISO/CEN	Peer reviewed	project reports
8.1 Skin irritation or skin corrosion, In vitro	Annex VII	Irritation • TG 439: In vitro skin irritation: Reconstructed Human Epidermis Test Method (RHE) Corrosion • TG 430: Transcutaneous Electrical Resistance Test Method (TER), • TG 431: In vitro skin corrosion: Reconstructed Human Epidermis Test Method (RHE) • TG 435: Corrositex - In Vitro Membrane Barrier Test Method			
8.3 Skin sensitization	Annex VII	In vitro: • TG 442D: In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (Keratinosens) • TG442E: In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (LuSens) • Update of TG 442D on in vitro skin sensitisation using animal-free serum and validation of TG 442E using human serum and human antibodies (Project4.118 – UK) • Detailed Review Paper on the Applicability of the key event based Test Guideline 442D for in vitro skin sensitisation testing of nanomaterials (Project 4.133 – SUI)			

		In Chemico: • TG 442C: In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)			
8.4. In Vivo gene mutation study Genotoxicity, In vivo	Annex IX	• TG 488: Transgenic rodent (TGR) somatic and germ cell gene mutation assays • TG 489: In vivo mammalian alkaline comet assay • New Guidance Document on the Adaptation of In vitro Mammalian Cell Based Genotoxicity TGs for Testing of Manufactured Nanomaterial (Project 4.95 – EC)			
8.4.2. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study	Annex VIII	• TG 487: In Vitro Mammalian Cell Micronucleus Test • TG 490: In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene			
8. 6. Repeated dose toxicity	Annex VIII	•			
8.6.1. Short-term repeated dose toxicity study (28 days), one species male and female, most appropriate route of human exposure	Annex VIII	• TG 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents			
8.6.2 Sub-chronic toxicity study (90-day), one species, rodent, male and female, most appropriate route of administration, having regard to the likely route of human exposure Inhalation and link to toxicokinetics	Annex IX	• TG 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents			
8.8.1 Assessment of the toxicokinetic behaviour of the substance to the extent that can be derived from the relevant available information Toxicokinetics	Annex VIII		ISO/TR 22019:2019 Nanotechnologies — Considerations for performing toxicokinetic studies with nanomaterials		

Table 4: REACH Endpoints environmental fate and ecotoxicity

Requirement/endpoint in REACH (2018)	REACH Annex	OECD TG/GD/report	Standard ISO/CEN	Peer reviewed	project reports
9.1 Aquatic toxicity	Annex VII	•			
9.1.1. Short-term toxicity testing on invertebrates (preferred species Daphnia)	Annex VII	• *TG 202: Daphnia sp., Acute Immobilisation Test.* • GD on Aquatic (and Sediment) Toxicity Testing of Nanomaterials (Project 2.51 – CA/US)			
9.1.2. Growth inhibition study aquatic plants (algae preferred)	Annex VII	• *TG 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test.* • *TG 221: Lemna sp. Growth Inhibition Test* • GD on Aquatic (and Sediment) Toxicity Testing of Nanomaterials (Project 2.51 – CA/US)			
9.1.3. Short-term toxicity testing on fish	Annex VII	• *TG 203: Fish, Acute Toxicity Test* • GD on Aquatic (and Sediment) Toxicity Testing of Nanomaterials (Project 2.51 – CA/US)			
9.1.5. Long-term toxicity testing on invertebrates (preferred species Daphnia)	Annex VII	• *TG 211: Daphnia magna Reproduction Test* • GD on Aquatic (and Sediment) Toxicity Testing of Nanomaterials (Project 2.51 – CA/US)			
9.1.6. Long-term toxicity testing on fish		• *TG 210: Fish, Early-life Stage Toxicity Test* • *TG 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages* • *TG 215: Fish, Juvenile Growth Test* • GD on Aquatic (and Sediment) Toxicity Testing of Nanomaterials (Project 2.51 – CA/US)			
9.2 Degradation • REACH (2018/1881): For nanoforms that are not soluble, nor have high dissolution rate, such test(s) shall consider morphological transformation (e.g. irreversible changes in particle size, shape and surface properties, loss of coating), chemical transformation (e.g. oxidation, reduction) and other abiotic degradation (e.g. photolysis).	Annex VII	• Generally not applicable for inorganic chemicals. • New GD on Aquatic (Environmental) Transformation of Nanomaterials (Project 3.16 – AT). This Guidance Document aims to cover abiotic core transformation and coating degradation.			
9.2.1. Biotic	Annex VII	•			
9.2.1.1. Ready biodegradability	Annex VII	•			

Requirement/endpoint in REACH (2018)	REACH Annex	OECD TG/GD/report	Standard ISO/CEN	Peer reviewed	project reports
9.2.1.2. Simulation testing on ultimate degradation in surface water (Bio)Degradation /Transformation	Annex VII	•			
9.3 Fate and behaviour in the environment	Annex VIII	• TG 318 - Dispersion Stability of Nanomaterials in Simulated Environmental Media • New TG on dissolution rate of nanomaterials in aquatic environment (Project 3.10 – US) • New GD (Decision-Tree) on agglomeration and dissolution behaviour of nanomaterials in aquatic media (Project 3.09 – DE)			
9.3.1. Adsorption / desorption screening (Bio)Degradation /Transformation	Annex VIII	• New GD on Aquatic (Environmental) Transformation of Nanomaterials (Project 3.16 – AT) • New GD to support implementation of TG312 'Leaching in Soil Columns' for Nanomaterial Safety Testing (Project 3.14 – DE/CA)			
9.3.2 Bioaccumulation in aquatic species, preferably fish	Annex IX	• New GD on Assessing the Apparent Accumulation Potential for Nanomaterials (TG305) (Project 3.12 – SP)			
9.3.3 Further information on adsorption desorption depending on the results of the study required in Annex VIII	Annex IX	• New GD to support implementation of TG312 'Leaching in Soil Columns' for Nanomaterial Safety Testing (Project 3.14 – DE/CA) • New TG on Manufactured Nanomaterial Removal in Wastewater Treatment Plants (Project 3.11 – US)			

[1] http://www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm