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Insights into possibilities for grouping and read-across for nanomaterials in EU chemicals legislation

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ABSTRACT

This paper presents a comprehensive review of European Union (EU) legislation addressing the safety of chemical substances, and possibilities within each piece of legislation for applying grouping and read-across approaches for the assessment of nanomaterials (NMs). Hence, this review considers both the overarching regulation of chemical substances under REACH (Regulation (EC) No 1907/2006 on registration, evaluation, authorization, and restriction of chemicals) and CLP (Regulation (EC) No 1272/2008 on classification, labeling and packaging of substances and mixtures) and the sector-specific pieces of legislation for cosmetic, plant protection and biocidal products, and legislation addressing food, novel food, and food contact materials. The relevant supporting documents (e.g. guidance documents) regarding each piece of legislation were identified and reviewed, considering the relevant technical and scientific literature. Prospective regulatory needs for implementing grouping in the assessment of NMs were identified, and the question whether each particular piece of legislation permits the use of grouping and read-across to address information gaps was answered.

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1. Introduction

The European Commission views nanotechnologies as Key Enabling Technologies (EC 2005), emphasizing the importance of also developing and establishing methodologies for assessing the safety of NMs. Risk assessment is based on the paradigm that risk can be expressed by combining information about hazard and exposure. A large number of NMs may share the same chemical composition but differ, for example, in particle size distribution, shape, and surface chemistry as suggested by the European Chemicals Agency, ECHA (ECHA 2017a), possibly resulting in different exposure, toxicokinetic and hazard profiles. Hence, regulators are discussing how to implement nanospecific grouping concepts within existing legislation, and guidance is needed on when and how to apply grouping to nanoforms ('nanoforms', see section 3.1). In current European Union (EU) chemicals legislation (EC 2006), adequately justified grouping and read-across between chemical substances is accepted for fulfilling information requirements for risk assessment. In

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general, research has improved the understanding of the type of NM characteristics, and other relevant issues for the grouping and read-across of NMs that are needed for a scientifically sound grouping (Sellers et al. 2015), also when it is aimed at enabling risk assessment (Dekkers et al. 2016). However, scientifically based grouping and read-across methods have not yet been fully established or accepted for NMs, though several science-based approaches for grouping NMs have been suggested (e.g. Arts et al. 2014; Arts et al. 2015; ECHA 2016a; Hund-Rinke, Nickel, and Kühnel 2017). As a first step, ECHA has proposed guidance relevant to NMs for grouping and read-across within one substance registration (ECHA 2017b).

Grouping and read-across of NMs may be performed for various other purposes in addition to that of filling data gaps and use in weight-of-evidence approaches (Arts et al. 2014; OECD 2016a), such as (a) supporting prioritization and selection of NMs for further testing; (b) enabling ranking of NMs according to selected endpoints; and (c) justifying waiving of specific tests for individual NMs. Establishing sound grouping concepts also improves the understanding of structure/property-activity relationships for NMs, and thus supports more targeted testing and risk assessment. Grouping may also be introduced into a Safe(r)-by-Design concept for design and manufacturing processes, thereby facilitating and targeting safety assessment at the design stage.

The NanoReg2 project, entitled 'Development and implementation of Grouping and Safe-by-Design approaches within regulatory frameworks', aims to develop scientifically based grouping approaches for NMs relevant for regulatory purposes and to establish a Safe(r)-by-Design approach for NMs.

Based on information gathered within NanoReg2 from peer-reviewed publications, websites of the European Commission including the Official Journal (OJ) of the European Union, European Agencies and Authorities, national governments, and national and international organizations (especially the Organisation for Economic Co-operation and Development, OECD), this paper presents a comprehensive overview of the possibilities, within different EU legislation addressing chemicals, for applying grouping and read-across for assessing NMs. Legislation addressing chemicals in the EU may either broadly cover almost all chemicals (horizontal legislation, e.g. REACH), or cover a specific use of chemicals (sector-specific legislation), see Table 1. Sector-specific legislation may also have to fulfill requirements from horizontal legislation. Each piece of legislation may have different requirements for applying grouping and read-across.

The concepts of grouping and read-across as defined and used by OECD and by ECHA (see below) are presented in this paper.

The paper highlights the following issues regarding each piece of legislation:

i. whether NMs are explicitly included in this specific piece of the legislation,
ii. whether it is considered appropriate to apply grouping and read-across to fulfill information requirements on a substance,
iii. whether it is considered appropriate to apply grouping and read-across for fulfilling information requirements on NMs, and
iv. what the specific needs for applying grouping and read-across to NMs are.

2. General considerations by OECD and ECHA on the grouping of chemicals

Grouping of chemicals is well established and widely applied. Specific guidelines and informative documents are available from the OECD (OECD 2014) and ECHA (ECHA 2008, 2012a, 2013a, 2014a, 2017a, 2017b, 2017c). The guidance defines two approaches for grouping and read-across between different substances: the analog and the category approach (definitions given in Box 1).

Box 1. Definitions of analog and category approach by the OECD and by ECHA

A) Analogue approach

The **OECD** definition (OECD 2014):

'When the focus of the assessment is on filling data gaps for one specific chemical, empirical data from one or more similar chemical(s) ("the analogue(s)") or "source" chemical(s)) can be used to predict the same endpoint for the "target" chemical, which is considered to be "similar". This analog approach is useful when the target and source chemicals share a known common mode (and/or mechanism) of action, and the adverse effects resulting from this mode (and/or mechanism) of action is evaluated. The analog approach could also be used in the absence of effects or when no specific mode (and/or mechanism) of action is expected and toxicokinetic behavior is not expected to differ significantly. In such case, more evidence, or more lines of evidence, should support the assessment'.

The **ECHA** definition (ECHA 2017b):

'The term "analog approach" is used when read-across is employed between a small number of structurally similar

substances; there is no trend or regular pattern on the properties. As a result of the structural similarity, a given toxicological property of one substance (the source) is used to predict the same property for another substance (the target) to fulfill a REACH information requirement'.

B) Category approach

The **OECD** definition (OECD 2014):

'Chemicals whose physical-chemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or "category" of chemicals. The assessment of chemicals by using this category approach differs from the approach of assessing them on an individual basis, since the properties of the individual chemicals within a category are assessed on the basis of the evaluation of the category as a whole, rather than based on measured data for any one particular chemical alone'.

ECHA definition (ECHA 2017b):

'The term "category approach" is used when read-across is employed between several substances that have structural similarity. These substances are grouped together on the basis of defined structural similarity and differences between the substances. As a result of the structural similarity, the toxicological, ecotoxicological, or environmental fate properties will either all be similar or follow a regular pattern. Predictions should cover all parameters as required in the respective REACH information requirements. It may be possible to make predictions within the group for the target substance(s) on the basis of a demonstrable regular pattern. Alternatively, whenever there is more than one source substance in the category and no regular pattern is demonstrated for the property under consideration, the prediction may be based on a read-across from a category member with relevant information in a conservative manner (worst case). The basis for the prediction must be explicit'.

According to the **OECD** (OECD 2014), the rationale of the analog or the category approach may be based on the following:

- Common functional group(s) (e.g., aldehyde, epoxide, ester, and specific metal ion);
- A common mode or mechanism of action or adverse outcome pathway;
- Common constituents or chemical classes, e.g. similar carbon range numbers. This is frequently applied with complex substances often known as 'substances of unknown or variable composition, complex reaction products or biological material' (UVCB substances);
- The likelihood of common precursors or breakdown products via physical or biological processes that result in structurally similar chemicals (e.g. the 'metabolic pathway approach' of examining related chemicals such as acid/ester/salt); or
- An incremental and constant change across the category (e.g. a chain-length category), often observed in physical chemical properties, for example, boiling point range.

According to **ECHA** (ECHA 2017b), structural similarity is a prerequisite for any grouping and read-across approach under REACH. These similarities may be due to a number of factors (REACH, Annex XI, 1.5):

- Common precursors or likelihood of common breakdown products via physical and/or biological processes which result in structurally-similar degradation products (i.e. similarity through bio-transformation); or
- A constant pattern in the changing of the potency of the properties across the group (i.e. of physico-chemical or biological properties).

Ideally, a category of substances should be based on more than one common feature to increase confidence in the validity of the category. Within a category, data gaps can be filled by, for example, trend analysis, (quantitative) structure-activity relationship, models, or read-across. The category approach can improve the assessment of compounds compared with the individual assessment of each compound while avoiding testing.

Scientifically valid and robust read-across requires a clear rationale for the grouping. The selection of analogs needs to be justified by general (i.e., physico-chemical) similarity and/or endpoint-specific considerations (e.g., biological similarity). When indications of an adverse effect are observed, grouping is more reliable if based on an at least partially known mode of action underlying that effect. Toxicokinetic information provides valuable mechanistic insights, and thus can be used to support grouping approaches. At present, concepts are being developed to improve the integration of mechanistic information, such as the adverse outcome pathways concept, which help to identify key steps in the chain of events leading to the effect (Kleinstreuer et al. 2016). Key events are applicable to alternative test methods, such as *in vitro*-based high-throughput screening, and may serve as indicators of adverse effects (Grafström et al. 2015). ECHA held a Topical Scientific Workshop on New Approach Methodologies in Regulatory Science with the objective of initiating a dialog on how New Approach Methodologies can support regulatory acceptance of grouping and read-across (ECHA 2016a); New Approach Methodologies data includes, for example, high-throughput screening and omics data, and it may be used as a rationale and to confirm the mechanistic hypotheses for read-across.

3. General considerations on grouping of nanomaterials

ECHA recently published a guidance document on the grouping of NMs (ECHA 2017c). OECD is currently developing principles and guidance for grouping of NMs (OECD 2016b) based on the relevant OECD guidance for chemicals (OECD 2014).

According to ECHA, either overall similarity (all tests for one form are representative of the other form(s)) or similarity regarding a specific endpoint, property or test result can be claimed between nanoforms and, where applicable, non-nanoform(s). The current ECHA discussion focusses on the

Table 1. Overview of legislation for chemicals, biocides, plant protection, cosmetics and food, including how grouping is addressed. ("Risk Assessment" refers to the area of risk assessment addressed under the legislation, which may be HH and/or ENV. HH: Human Health, ENV: Environment, and Fate and behavior, YES: the column header is reflected in the legal text, NO: the column header is not reflected in the legal text, n/a: not applicable).

Area of application legislation	Nano-definition (legal text)/Other information	Grouping explicitly mentioned in legal text	Read-across possible as an alternative method[1]	Nano-content labeling	Guidance for grouping	Guidance for grouping of NMs	Risk assessment	
							HH	ENV
REACH (EC 2006)	The definition of the term 'substance' implicitly covers all forms at any size. Amended REACH annexes (EC 2018) have been proposed, in which the term 'nanoform' is defined. ECHA applies the EC NM Definition for REACH registration purposes (1). Annexes are currently under review to include a definition and nanospecific requirements.	Annex XI	YES	NO	YES	YES	YES	YES
CLP (EC 2008a)	No definition	NO	YES	NO	YES	NO	YES	YES
Sector specific legislation								
Biocidal Products (EU 2012a)	*Article 3* **Definitions** (z) 'nanomaterial' means a natural or manufactured active substance or non-active substance containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm. Fullerenes, graphene flakes and single-wall carbon nanotubes with one or more external dimensions below 1 nm shall be considered as nanomaterials. For the purposes of the definition of nanomaterial, 'particle', 'agglomerate' and 'aggregate' are defined as follows: — 'particle' means a minute piece of matter with defined physical boundaries, — 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components, — 'aggregate' means a particle comprising strongly bound or fused particles;	YES	YES	YES	YES	NO	YES	YES
Plant protection products (EC 2009a)	No definition	NO	YES (Not for food residues)	NO	YES (For some aspects of the assessment)	NO	YES	YES
Cosmetic products (EC 2009b)	*Article 2* **Definitions** (k) an insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.	NO	YES	YES	NO. Reference to OECD guidance	NO	YES	YES (under REACH)
Food contact materials (EC 2004)	No definition	NO	YES	NO	YES	NO	YES	
Plastic food contact materials (EU 2011a)	No definition Regulation (EU) No 10/2011 provides some specifications for engineered NMs: Recital 23 'New technologies engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example,	NO	YES –but explicitly excluded for nanomaterials	NO	NO	NO	YES	

(*continued*)

Table 1. Continued.

Area of application legislation	Nano-definition (legal text)/Other information	Grouping explicitly mentioned in legal text	Read-across possible as an alternative method[1]	Nano-content labeling	Guidance for grouping	Guidance for grouping of NMs	Risk assessment	
							HH	ENV
	nanoparticles. These different properties may lead to different toxicological properties and therefore these substances should be assessed on a case-by-case basis by the Authority as regards their risk until more information is known about such new technology. Therefore it should be made clear that authorizations which are based on the risk assessment of the conventional particle size of a substance do not cover engineered nanoparticles'. Recital 27: In recent years, plastic food contact materials are being developed that do not only consist of one plastic but combine up to 15 different plastic layers to attain optimum functionality and protection of the food, while reducing packaging waste. In such a plastic multi-layer material or article, layers may be separated from the food by a functional barrier. This barrier is a layer within food contact materials or articles preventing migration of substances from behind that barrier into the food. Behind a functional barrier, non-authorized substances may be used, provided they fulfill certain criteria and their migration remains below a given detection limit … … .New technologies that engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles, should be assessed on a case-by-case basis as regards their risk until more information is known about such new technology. Therefore, they should not be covered by the functional barrier concept. *Article 9* **Specific requirements on substances** (2) Substances in nanoform shall only be used if explicitly authorized and mentioned in the specifications in Annex I. *Article 13* **Plastic multi-layer materials and articles** 4. The substances not listed in the Union list or provisional list referred to in paragraph 2(b) shall not belong to either of the following categories: (a) substances classified as 'mutagenic', 'carcinogenic', or 'toxic to reproduction' in accordance with the criteria set out in sections 3.5, 3.6. and 3.7 of Annex I to Regulation (EC)No 1272/2008 of the European Parliament and the Council(1) OJ L 353, 31.12.2008, p. 1.; (b) substances in nanoform. *Article 14* **Multi-material multi-layer materials and articles** 1. In a multi-material multi-layer material or article, the							

(*continued*)

Table 1. Continued.

Area of application legislation	Nano-definition (legal text)/Other information	Grouping explicitly mentioned in legal text	Read-across possible as an alternative method[1]	Nano-content labeling	Guidance for grouping	Guidance for grouping of NMs	Risk assessment	
							HH	ENV
	composition of each plastic layer shall comply with this Regulation. 2. By derogation from paragraph 1, in a multi-material multi-layer material or article a plastic layer which is not in direct contact with food and is separated from the food by a functional barrier, may be manufactured with substances not listed in the Union list or the provisional list.3. The substances not listed in the Union list or provisional list referred to in paragraph two shall not belong to either of the following categories: (a) substances classified as 'mutagenic', 'carcinogenic' or 'toxic to reproduction' in accordance with the criteria set out in sections 3.5, 3.6. and 3.7 of Annex I to Regulation (EC)No 1272/2008; b) substances in nanoform.							
Active and intelligent food contact materials and articles (EC 2009c).	No definition Recital 14 states: '… New technologies that engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles, should be assessed on a case-by-case basis as regards their risk until more information is known about such new technology. …' *Article 5* **Community list of substances that may be used in active and intelligent components** 1. Only substances which are included in the 'Community list' of authorized substances (hereinafter referred to as the Community list) may be used in components of active and intelligent materials and articles. 2. By way of derogation from paragraph 1, the following substances may be used in components of active and intelligent materials and articles without being included in the Community list: … (c) substances used in components which are not in direct contact with food or the environment surrounding the food and are separated from the food by a functional barrier provided that they comply with the conditions set out in Article 10 and that they do not fall within either of the following categories: … (ii) substances deliberately engineered to particle size which exhibit functional physical and chemical properties that significantly differ from those at a larger scale.	NO	NO	NO	NO	NO	YES	
Novel foods (EU 2015b)	*Article 3* **Definitions** (f) 'engineered nanomaterial' means any intentionally produced	NO		YES (EU) No 1169/2011	NO	NO	YES	

(*continued*)

Table 1. Continued.

Area of application legislation	Nano-definition (legal text)/Other information	Grouping explicitly mentioned in legal text	Read-across possible as an alternative method[1]	Nano-content labeling	Guidance for grouping	Guidance for grouping of NMs	Risk assessment	
							HH	ENV
	material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. Properties that are characteristic of the nanoscale include: (i) those related to the large specific surface area of the materials considered; and/or (ii) specific physico-chemical properties that are different from those of the non-nanoform of the same material.							
Food additives (EC 2008b)	No definition Other information: *Article 12* **Changes in the production process or starting materials of a food additive already included in a Community list** When a food additive is already included in a Community list and there is a significant change in its production methods or in the starting materials used, or there is a change in particle size, for example through nanotechnology, the food additive prepared by those new methods or materials shall be considered as a different additive and a new entry in the Community lists or a change in the specifications shall be required before it can be placed on the market.	NO	YES	YES (EU) No 1169/2011	NO	NO	YES	
Food enzymes (EC 2008c)	No definition Other information: Recital12 A food enzyme already included in the Community list under this Regulation which is prepared by production methods or using starting materials significantly different from those included in the risk assessment of the Authority, or different from those covered by the authorization and the specifications under this Regulation, should be submitted for evaluation by the Authority. 'Significantly different' could mean inter alia a change of the production method from extraction from a plant to production by fermentation using a micro-organism or a genetic modification of the original micro-organism, a change in starting materials, or a change in particle size.	NO	NO	YES (EU) No 1169/2011	NO	NO	YES	
Flavourings (EC 2008d)	No definition	NO	YES	YES (EU) No 1169/2011	NO	NO	YES	
Food supplements (EC 2002)	No definition Food Supplements that are also Novel Foods should are covered by both legislations, see requirements for Novel Foods.	NO	NO	NO	NO	NO	YES	
Food intended for infants and young children, food	Recital 23 When there is a significant change in the production method of	NO	NO	NO	NO	NO	YES	

(*continued*)

Table 1. Continued.

Area of application legislation	Nano-definition (legal text)/Other information	Grouping explicitly mentioned in legal text	Read-across possible as an alternative method[1]	Nano-content labeling	Guidance for grouping	Guidance for grouping of NMs	Risk assessment	
							HH	ENV
for special medical purposes, and total diet replacement for weight control (EU 2013)	a substance that has been used in accordance with this Regulation or a change in particle size of such a substance, for example through nanotechnology, that substance should be considered different from the one that has been used in accordance with this Regulation and should be re-evaluated under Regulation (EC) No 258/97 and subsequently under this Regulation. *Article 2* **Definitions** 1. For the purposes of this Regulation, the following definitions shall apply: (b) the definitions of 'prepacked food', 'labeling' and 'engineered nanomaterial' set out respectively in points (e), (j) and (t) of Article 2(2) of Regulation (EU) No 1169/2011; Other information: *Article 9* **General compositional and information requirements** … 2. Food referred to in Article 1(1) shall not contain any substance in such quantity as to endanger the health of the persons for whom it is intended. For substances which are engineered nanomaterials, compliance with the requirement referred to in the first subparagraph shall be demonstrated on the basis of adequate test methods, where appropriate.							
Food information to EU (2011b)	Same as (EU) 2015/2283 Other Information: *Article 18* **List of ingredients** All ingredients present in the form of engineered nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word 'nano' in brackets.	n/a	n/a	YES	n/a	n/a	n/a	n/a

grouping of nanoforms within the same substance registration.

There are multiple reviews of the state of the art for the grouping of NMs (Arts et al. 2014, 2015; ECHA 2016a). Arts et al. (2014) focussed on human health aspects (specifically, inhalation), and proposed that the apical toxic effects of NMs are directed by intrinsic material properties and extrinsic properties (Arts et al. 2015), that is, properties that depend on the surroundings. However, neither the exact correlation(s) of properties and effects nor the interdependence of some material properties are yet established; thus, the grouping of NMs should not rely on intrinsic material properties alone. Also extrinsic properties (which remain to be validated) could be important, for example, biological interactions, dissolution under relevant (physiological) conditions, biokinetics, uptake and distribution, early and apical biological effects of the NMs (e.g., *in vitro*), and life-cycle aspects as well as specific uses and exposure scenarios.

The OECD has organized two expert meetings to develop grouping approaches for NMs, giving an overview of existing approaches (OECD 2016a). The background information included a survey (OECD 2016c) to assess whether and how concepts of grouping, equivalence, and read-across based on physico-chemical properties have already been used in hazard assessment of NMs under different regulatory frameworks. The second expert meeting (OECD 2016b) aimed at a common understanding of aspects to be considered when applying grouping and read-across in the regulatory hazard assessment of NMs, and to provide initial input for a future update of Section 6.9 of the OECD Guidance (OECD 2014).

3.1. Registration, evaluation, authorisation and restriction of chemicals (REACH)

REACH (EC 2006) defines substance as 'a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition'. Also NMs fall under this definition. The current text of REACH does not explicitly address NMs, nor does it state any specific requirements for NMs; amendments to the REACH Annexes to specifically address information requirements for NMs have been agreed upon (EC 2018), and an update of the regulatory definition of NMs to be applied in EU legislation is under discussion.

ECHA applies the definition of NM recommended by EC (EC 2011) and has developed a 'best practice' document to define the term 'nanoform' (ECHA 2017a) for the purposes of REACH and CLP, to distinguish different NMs of the same chemical composition. The agreed amendments of the REACH Annexes include this term and defines 'nanoform' as a form of a substance that fulfills the definition of a NM and that is further distinguished from other forms based on differences in size distribution, shape, surface area, and surface chemistry (EC 2018). By 4 June 2018, ECHA's website indicated 26 substances having a nanoform (ECHA 2016b). This low number is probably partially due to regulatory uncertainty, as there is currently no legal requirement to state whether a substance registration includes nanoforms.

REACH registration is based on the 'one substance, one registration' principle; thus one registration dossier contains data on one substance, and the information provided should be adequate for assessing the risk of all form(s) of the substance (e.g. non-nanoforms, nanoforms).

In 2012, ECHA published appendices updating some chapters of the Guidance on Information Requirements and Chemical Safety Assessment with information relevant to NMs (ECHA 2012b, 2012c, 2012d). A further update in 2017 (ECHA 2017c, 2017d, 2017e, 2017f) provided specific recommendations for NMs on how to meet the REACH information requirements and use relevant hazard data, including specific recommendations on grouping and read-across for NMs (ECHA 2017c). It also promotes an enhanced scientific understanding of the hazard and risk assessment of NMs. ECHA has also updated the International Uniform Chemical Information Database, IUCLID, and its user manual (ECHA 2017g) according to OECD Harmonized Templates 101–113, available at https://www.oecd.org/ehs/templates/, which are designed to collect information specifically relevant to NMs, and

enabled registrants to explicitly report composition and test results for nanoforms.

Several unresolved issues remain, in particular the adequate physico-chemical characterization of nanoform(s) (Rasmussen et al. 2018). Furthermore, clarification is needed regarding what specific data for NMs is necessary to fulfill the (new) REACH information requirements. In parallel, the suitability of test guidelines for NMs needs to be confirmed and, if necessary, test guidelines need to be adapted to NMs, or new ones may have to be developed (OECD 2009). Other challenges include issues of substance identity and the coverage of morphological variants of nanoforms in registrations, the impact of surface modification on substance identity, and how core-shell constructions or doped materials should be addressed. Recently, requirements for NMs were agreed upon in the amendments of the REACH Annexes (EC 2018).

Alternative methods, including grouping and read-across, are encouraged under REACH. Annex XI, 1.5 of REACH specifically refers to grouping and read-across between substances, which may be considered '*when substances have structural similarities and results are adequate for the purpose of use, have adequate and reliable coverage of the key parameters addressed in the corresponding test method, cover exposure duration comparable to or longer than the corresponding test method, and are adequately and reliably documented*'. Applying these methods under REACH is supported by the guidance for grouping of chemicals for registration purposes (ECHA 2008) and the Read-Across Assessment Framework (ECHA 2017b) for the consistent evaluation by ECHA of the scientific aspects of read-across. The role of the Read-Across Assessment Framework in assisting the application of New Approach Methodologies for weight-of-evidence approaches and in decreasing uncertainty in read-across is currently being explored (ECHA 2016a) and further addressed in the agreed amendments of the REACH Annexes (EC 2018).

As REACH covers NMs, grouping and read-across for fulfilling information requirements for NMs is applicable in principle. ECHA has published a 'best practice' document for the identification and characterization of NMs (ECHA 2017a), which partly builds on two previous 'best practice reports' (ECHA 2013b, 2014a) from the ECHA Group Assessing Already Registered Nanomaterials (GAARN). These reports recommend applying the general similarity rules (i.e. 'criteria') mentioned in Annex XI of REACH to NMs but suggest that in addition to chemical composition, more information about physico-chemical parameters (e.g. aspect ratio, shape, solubility, surface area, charge, and surface treatment) is needed to support claims of similarity (or differences) between nanoforms or nano and non-nano form(s). The agreed amendment of the REACH Annex XI follows this line, stating that 'for grouping different nanoforms of the same substance the molecular structural similarities alone cannot serve as a justification' (EC 2018). As with other chemicals, the grouping of NMs should be justified by more than one basis or criterion. The endpoint(s) and route(s) of exposure addressed and whether the grouping allows quantitative or only qualitative assessment need to be specified. Furthermore, it is recommended to obtain knowledge of the nanoforms' biokinetics to establish grouping of NMs.

The appendix to the Guidance on Quantitative Structure-Activity Relationships and Grouping, providing recommendations for NMs (ECHA 2017c), proposes performing grouping and read-across of NMs only within one substance identity. The outlined iterative procedure comprises the following steps: (i) identifying nanoforms of a given substance considered relevant for the grouping, (ii) establishing a grouping hypothesis, performing an initial grouping and identifying the nanoforms within the group, (iii) collecting all available information in a data matrix, and (iv) assessing the group, and identifying and filling data gaps. Importantly, the robustness of the grouping and the validity of the grouping hypothesis must be assessed at this stage.

A main building block (ECHA et al. 2016) for the above-mentioned guidance describes a strategy for read-across for NMs. It notes that similarity claims should be based on physico-chemical parameters and that a read-across hypothesis should be scientifically justified, for example, by substantiating how the physico-chemical parameters affect toxicokinetic behavior so that less of the target nanoform reaches the target site, or by substantiating that the target nanoform is less hazardous (ECHA et al. 2016, Oomen et al. 2015). The document summarizes the parameters relevant for describing the physical and chemical identity of NMs and their (eco)toxicity,

toxicokinetics, and environmental fate. The parameters used to demonstrate similarity can vary among different NMs, and properties may change during the life cycle of the material. Several physico-chemical parameters are interconnected, and some of them influence toxicity directly, others indirectly through toxicokinetics.

So far, when registering nanoforms, data from the registration dossier referring to the non-nanoform of the substance or other nanoforms have been used to fill data gaps. The 2018 registration of substances (at 1–100 t/y) is, under the assumption that a large proportion of NMs and substances with nanoforms are manufactured or imported at this tonnage level, expected to increase the number of registered nanoforms.

The present practice under REACH shows a need for reducing regulatory uncertainty regarding the application of grouping and read-across to NMs. A general concept for grouping nanoforms is available (ECHA 2017c), but further practical experience and guidance are needed to apply robust regulatory grouping and read-across between different nanoforms or non-nanoform(s) and nanoform(s) of a substance. Currently, addressing substance identity issues under REACH, including the identification and characterization of the different nanoforms within a registration, is particularly challenging. Besides structural similarity, relevant and unequivocal criteria (and guidance) for claiming similarity between different nanoforms or between nanoform(s) and non-nanoform(s) of a substance are needed. ECHA has recently established well-defined criteria for distinguishing different nanoforms within a single dossier (ECHA 2017a). A number of proof-of-principle case studies are needed to gain further insight into these issues.

3.2. Classification, labelling and packaging (CLP)

The definition of 'substance' under CLP (EC 2008a) is identical to that under REACH; therefore, NMs are also covered by CLP. According to its article 5, CLP applies to substances and mixtures in all physical states and forms and recognizes that different forms of a substance or mixture may require different classifications. The 'Guidance on the Application of the CLP Criteria' (ECHA 2017h) does not explicitly address NMs, but the influence of relevant properties on test results for fine powders (e.g. particle size, specific surface area, and shape) is discussed for each endpoint, thus implicitly addressing the classification of NMs. For substance classification, some physico-chemical data must be provided, but regarding (eco)toxicity information, only the existing information is needed. Data may be generated by using test methods advised by REACH.

The Nanomaterials Informal Correspondence Group under the United Nations is currently discussing nanomaterial-related amendments at United Nations level. This includes topics such as whether 'dust', 'particles', and 'powders' in the Globally Harmonized System adequately cover NMs, which units/metrics for classification and cutoff values are applicable to NMs, and which non-testing approaches are applicable and needed. Other topics include the adequate reporting of nanoforms in Safety Data Sheets and the appropriateness of group entries for NMs.

CLP states that manufacturers, importers, and downstream users of a substance must identify the relevant available information for determining whether the substance causes a physical, health, or environmental hazard, including '*any other information generated in accordance with section 1 of Annex XI to Regulation (EC) No 1907/2006 (REACH)*'. This REACH Annex describes the 'General rules for adaptation of the standard testing regime set out in Annexes VII to X', including definitions for grouping of substances and read-across approaches. Accordingly, grouping and read-across is applicable to NMs under CLP. Different nanoforms of the same substance may be classified differently depending on their specific hazard profile (EC, 2008a).

When applying grouping and read-across under CLP, the REACH provisions apply. Thus, the challenges and needs identified for REACH should be considered. The 'Guidance on the Application of the CLP Criteria' (ECHA 2017h) may require nano-specific adjustments, which are currently discussed at United Nations Globally Harmonized System level and by the Nanomaterials Expert Group at ECHA.

3.3. Food contact materials

The framework regulation on food contact materials (FCMs) (EC 2004) does not specifically address NMs.

However, the regulation on plastic materials and articles intended to come into contact with food (EU 2011a) refers to engineered NMs in the recitals (see Table 1). Substances in nanoform are only allowed in plastic FCMs if they are explicitly authorized and entered onto the positive list, which currently includes titanium nitride nanoparticles (FCM No 807 (CEF 2012)) and carbon black (FCM No 411 (EU 2011a)). The specifications of kaolin (FCM No 410 (EU 2015a)) and silanated SiO_2 (FCM No 87 (EU 2016, CEF 2014a)) are extended to substances in the nanoform, and four co-polymers in nanoform (FCM No 859, 998, 1016, and 1043) are included, based on the rationale that no release has been detected (EU 2015a, CEF 2014a, 2014b). The European Food Safety Authority (EFSA) has also published an opinion on 'zinc oxide, nanoparticles', which are soluble, leading to an initial concern that the daily intake of zinc may be exceeded; however, 'zinc oxide, nanoparticles' have been authorized as FCM No 1046 (coated) and 1050 (uncoated) (EU 2016, CEF 2016a).

Food may also be protected by multi-layer FCMs containing functional barrier layers that ensure, for example, a reduction in the migration or no detectable migration of substances from the packaging material into food. If the functional barrier is in contact with the food, specific substance migration requirements have to be met. According to Regulation 10/2011 (EU 2011a), the functional barrier may protect the food from contact with chemicals. This barrier is a layer within FCMs or articles preventing the migration of substances from behind that barrier into the food. Behind a functional barrier, non-authorized substances may be used. This, however, does not apply to NMs (recital 27, Article 14).

The FCM regulation (EC 2004) does not exclude applying alternative methods to evaluating human health hazards of chemicals used in FCMs, and thus it should be possible to use grouping approaches and read-across in toxicological assessments. To date, read-across has been applied to certain endpoints for only a few non-nanoforms of substances (e.g. genotoxic potential of ethylene glycol dipalmitate and dipentaerythrol stearate) (CEF 2015a, 2015b 2016b).

EFSA guidance (EFSA 2011), which is currently being updated, states that the risk assessment of NMs in FCMs may use relevant information obtained by read-across from other manufactured NMs or non-nanoforms (i.e. larger-sized, molecular, and ionic forms). To date, however, all NMs have been evaluated case by case, as limited information is available on how the specific properties of NMs affect their release from FCMs or their toxicokinetic and toxicological profiles (EFSA 2016).

In general, article 9 of the regulation on plastic FCMs (EU 2011a) specifies that '*Substances in nanoform shall only be used if explicitly authorized and mentioned in the specifications in Annex I*'. This excludes grouping and read-across for NMs until more information is available about such new technology; consequently, risks of FCM substances in the nanoform must be assessed case by case by EFSA.

Grouping and read-across for the toxicological evaluation of NMs present in FCMs requires broad knowledge on how specific physico-chemical properties affect the migration or transport, toxicokinetic behavior, and toxicity of the material, which reduces the uncertainty related to toxicological evaluations based on grouping of NMs. EFSA has proposed guidance on the toxicological assessment of NMs (CEF 2016b), which may help to implement read-across approaches. For plastic FCMs, the regulation would need to be amended to allow grouping.

As migration testing of functional barrier materials is complex and resource intensive, compliance can be demonstrated also by other means, such as calculation, including modeling, and scientific evidence or reasoning, which can be software-assisted. Grouping may help to categorize functional barrier performances that depend on diffusion or migration parameters of substances in FCMs.

3.4. Food and novel foods

NMs are addressed in regulations covering novel foods (EU 2015b) and various types of additives to food (EC 2008b, 2008c, 2008d), referring either to the use of nanotechnology or to manufactured (engineered) NMs. The latter term is defined in the Novel Foods Regulation (EU 2015b) and used also in the regulation on Food Information to Consumers (EU 2011b), which requires that the labeling of food products indicates the presence of

manufactured NMs (see Table 1 for further details). The definition in the Novel Foods Regulation (EU 2015b) is different from the EC recommendation (EC 2011) and, for example, does not have a threshold for the number of particles at nanoscale, whereas the EC recommendation suggests a minimum content of 50% of the number of particles at nanoscale for a material to be a NM. In the risk assessment of substances in food, various alternative methods are allowed for filling in data gaps for specific endpoints, and read-across has been applied for some food additives and flavoring agents (e.g. methylbenzophenone (CEF 2009)).

The Regulation on Food Additives (EC 2008b) requires that substances modified through '*the use of nanotechnology*' are registered on the list of approved food additives as new substances.

Substances are assessed case by case, and a novel food intended for the market must comply with article 7 of the Novel Foods Regulation (EU 2015b), that is, it must not '*on the basis of the scientific evidence available, pose a safety risk to human health*'. For novel foods containing engineered NMs, '*an explanation shall be provided by the applicants of scientific appropriateness* [of the test methods] *for nanomaterials and, where applicable, of the technical adaptations or adjustments that have been made in order to respond to the specific characteristics of those materials*' (recitals of (EU 2015b)). For this reason, and as no specific guidance exists on grouping or read-across for substances in food and novel foods, these methods are not commonly used by EFSA in the assessment of NMs.

Engineered NMs in food and novel foods are assessed by EFSA on a case-by-case basis; however, EFSA's expert panels take all scientifically justified information into account, including data generated through grouping and read-across approaches.

In the report by the Scientific Network for Risk Assessment of Nanotechnologies in Food and Feed, EFSA informed the food and feed regulators of its efforts to **'***clarify concept and/or principles on how nanoforms can be "clustered" into different groups***'** (EFSA 2016). Furthermore, in its risk assessment guidance (EFSA 2011), EFSA states that adequate characterization is essential for establishing the identity and the physico-chemical forms of NMs in food/feed products and under testing conditions. When a NM completely dissolves or degrades in the gastro-intestinal tract, hazard identification and hazard characterization can rely on data for the non-nanoform substance (if available), as long as the possibility of NM absorption before the dissolution or degradation stage can be excluded. In the guidance, EFSA emphasizes the importance of physico-chemical characterization of the pristine material, test material, and related forms in food matrices as a contribution to the knowledge base which in the future can be used for extrapolation or read-across procedures.

The implementation of grouping and read-across under the food and novel foods legislation will depend on the development of reliable methodology and its implementation by EFSA for assessing individual novel foods containing engineered NMs.

3.5. Biocidal products regulation

The Biocidal Products Regulation (BPR) (EU 2012a) requires a two-step assessment procedure. Firstly, the active biocidal substances are approved at EU level and entered on a positive list for a time-limited period. Secondly, any products containing listed active substance(s) must be authorized either at Member State level by the national authority, or at EU level via ECHA. ECHA maintains an on-line register of all authorized biocidal products. BPR further specifies the conditions for classification, packaging and labeling of biocidal products, referring also to CLP (EC 2008a). Data requirements under BPR address both active substances and products and go beyond the dataset specified in REACH.

BPR was the first legal act to define NMs based on the EC Definition (EC 2011) (Table 1), applying the first part of the Definition, that is, limiting the threshold to 50% of the number of particles at nanoscale.

The approval of an active substance does not cover nanoforms unless explicitly mentioned. Where NMs are used in biocidal products '*as active substance and/or co-formulant, a dedicated risk assessment is needed*'. The simplified authorization procedure for biocidal products is not applicable to products containing NMs. The name of any NM present in a biocidal product must be clearly stated on the label, together with any specific, related risks.

When test methods for identifying hazards are applied to NMs or to products containing them, their scientific appropriateness for NMs and, as

relevant, the technical adaptations or adjustments made in response to the specific characteristics of NMs must be explained.

Under the BPR review program, some NMs have been approved for biocidal use, for example, pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide (EC 2017), and silicon dioxide (as a NM in the form of aggregates or agglomerates) (EC 2014). Silver adsorbed on silicon has been determined to be a NM (ECHA 2014b) and will be evaluated as well.

Annex IV of the BPR establishes the rules for cases where an applicant proposes to adapt data requirements, including cases where '*(a) the data are not necessary owing to the exposure associated with the proposed uses; (b) it is not scientifically necessary to supply the data; or (c) it is not technically possible to generate the data*'. Annex IV, paragraph 1.5 specifies the rules on grouping and read-across. The BPR and REACH provisions are almost identical. Thus, the considerations for REACH (see section 3.1) seem to apply also to BPR.

In principle, it is possible to use grouping and read-across for assessing NMs under BPR. However, the above-mentioned two nanoforms of silicon dioxide were approved (EC 2014, 2017) after individual evaluation based on data, not read-across.

Grouping is therefore possible, also for assessing the hazards of NMs, but there is no specific guidance for grouping of NMs under the BPR. As under REACH and CLP, scientifically sound specific approaches for grouping and read-across of NMs used in biocidal products remain to be developed.

3.6. Plant protection products regulation

The Plant Protection Products Regulation (PPPR (EC 2009a)) applies to plant protection products (PPPs), which consist of or contain active substances, safeners or synergists, and co-formulants. PPPs are, among other purposes, intended for protecting plants or plant products against harmful organisms; influencing the life processes of plants (e.g. growth), other than as a nutrient; preserving plant products; destroying undesired plants or plants parts; or checking or preventing undesired growth of plants. PPPR requires a two-step assessment procedure very similar to that under BPR (see section 3.5): firstly, active substances are assessed by EFSA's PPP Panel and if approved, entered on a positive list valid at EU level for a time-limited period. Products containing approved active substances are then authorized at Member State level or within one of three pre-defined climate regions. Data requirements under PPPR address both active substances and products and go beyond the dataset specified in REACH. Co-formulants must not exhibit harmful effects on human or animal health or the environment, and unacceptable co-formulants must be listed in Annex III of PPPR.

PPPR does not explicitly mention NMs. It requires a case-by-case risk assessment for all active substances, including those in the nanoform, and for all products, including products containing NMs.

PPPR does not exclude using alternative methods for the evaluation of PPPs. For products, toxicity data for the required endpoints are in practice often extrapolated from similar products, based on information on the active substance(s) and the co-formulants, to reduce the workload and animal testing (Kah et al. 2013).

Grouping and read-across should not replace conventional risk assessment in the evaluation of active substances as residues, for example, in food. These should be assessed prior to product authorization using the dossiers including toxicological tests (EC 2009**a**).

The risks of substances used in PPPs, including NMs, are assessed on a case-by-case basis within the approval and authorization procedures. As described above, a combination of (quantitative) structure-activity relationships and read-across models can be used, but these approaches should not be used as alternatives to the conventional risk assessment of residues of active substances occurring, e.g., in food.

The environmental and human health effects of very few nano-PPPs have so far been evaluated under PPPR (Aschberger et al. 2015). To implement grouping and read-across in the hazard assessment of NMs used in PPPs, guidance is needed, as are techniques and tools for characterizing the properties of nano-PPPs. Models addressing properties and processes relevant to NMs also need to be developed, since the current environmental exposure and effect models fail to consider particle size, functionalization, shapes, and surface properties of NMs or changes taking place in environmental matrices.

3.7. Cosmetic products regulation

The Cosmetic Products Regulation (CPR (EC 2009b)) prohibits the marketing of products containing ingredients or combinations of ingredients that have been subject to animal testing (unless obtained before July 2013 or generated for other legislation). The rules for the human health risk assessment of substances used in cosmetic products are laid down in the CPR, and their environmental risk assessment is performed under REACH.

The CPR from 2009 includes some new rules for the use of NMs in cosmetic products, e.g., any intended use of NMs in cosmetic products must be notified to the Commission at least six months prior to placing them on the market, except where they had already been placed on the market before 11 January 2013. In case the use of a NM causes concern, the Commission shall request a scientific opinion from the Scientific Committee on Consumer Safety (SCCS) on the safety of the proposed specific use of the NM. The SCCS opinion should be based on the full information made available by the applicant.

SCCS has published a Guidance on Risk Assessment of Nanomaterials (SCCS 2012a), which is currently being updated, as well as a checklist for applicants submitting dossiers on NMs as cosmetic ingredients (SCCS 2017a). The regularly updated SCCS 'Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation' also contain a section on NMs, the 9th revision having been published in 2016 (SCCS 2016a).

The CPR explicitly addresses NMs, defines 'nanomaterial' (Table 1), and states a procedure for the notification, labeling, and risk evaluation of cosmetic products containing NMs. The CPR NM definition covers NMs that are intentionally produced and are insoluble or biopersistent (e.g., metals, metal oxides, carbon materials), whereas it does not cover NMs which are either soluble or degradable, i.e., not persistent in biological systems (e.g., liposomes, oil/water emulsions).

SCCS recognizes that the physico-chemical properties, biokinetic behavior, and biological effects of nanoforms of substances may differ from those of the non-nanoforms. The risk assessment approach for NMs used in cosmetic products is described in SCCS (2015a), and more detailed guidance is given in SCCS (2012a, 2013a). The SCCS has published scientific opinions on several individual NMs (e.g., 1,3,5-Tirazine, 2,4,6-tris[1,1'-biphenyl]-4-yl (SCCS 2011); zinc oxide (SCCS 2012b); Silica, Hydrated Silica and Silica Surface Modified with Alkyl Silylates (SCCS 2015b); titanium dioxide (SCCS 2013b), titanium dioxide in spray (SCCS 2017b), carbon black (SCCS 2013c), MBBT (SCCS 2015c) and hydroxyapatite (SCCS 2015d)), providing examples of the scientific evidence required in a NM dossier.

The SCCS Guidance on Risk Assessment of Nanomaterials (SCCS 2012a) emphasizes that for cosmetic ingredients that are NMs, data is required from tests carried out especially considering the nanoscale properties, which includes producing detailed data on the identity and composition of the NM (or a justifiably comparable material) intended for use in the final product. This characterization must include measuring the physico-chemical parameters listed in the SCCS Guidance (SCCS 2012a) and needs to be carried out at the raw material stage, in the cosmetic formulation, and during exposure for toxicological evaluation. Where needed and to facilitate risk assessment, the SCCS may request further information. Furthermore, the required base set of data on toxicological endpoints under CPR must be submitted, and depending on the test results, additional information may be required.

For the risk evaluation of cosmetic ingredients, including NMs, all available scientific data (including that generated by alternative methods replacing animal tests) are considered, including the physical and chemical properties, *in silico* data such as results obtained from (quantitative) structure-activity relationship calculations, chemical categories, grouping, read-across, physiologically-based pharmacokinetic or toxicokinetic modeling, *in vitro* data, and data obtained from *in vivo* studies (obtained before July 2013).

In principle, read-across and grouping is possible under CPR if the underlying data is sufficiently robust to enable read-across and grouping. The SCCS has addressed the issue in its Memorandum on Relevance, Adequacy and Quality of Data in Safety Dossiers on Nanomaterials (SCCS 2013a), which states (section 1.8) that '*Unless there is a close similarity between different nanomaterials, it is advisable to include a complete set of supporting data on*

each nanomaterial, rather than presenting several different nanomaterials in a single, patchy, and data-poor submission. If more than one nanomaterial is included in the dossier, the basis for "close similarity" to allow data read-across between the nanomaterials must also be provided. This should not only relate to the chemical composition of the core nanomaterial, but also the physical/morphological features and other characteristics, such as surface coating or other modifications', and (section 1.4) '*For example, safety of a nanomaterial cannot be assumed on the argument that the bulk form of the materials is safe (and vice versa), without specific evidence to support it*'.

In silico methodology for chemicals has made great progress in the recent past (Teixeira do Amaral et al. 2014, SCCS 2016a). For NMs, data are still limited, and relationships between physico-chemical aspects and toxicological effects have not yet been established to allow the development of reliable and robust *in silico* models. Accordingly, *in silico* methods for read-across and grouping of NMs are in general not yet considered ready to be applied for regulatory purposes (SCCS 2012a).

The assessment of the use of titanium dioxide as an UV-filter in dermal cosmetic products could be considered an example of applying read-across (see overall conclusion of (SCCS 2013b, 2016b)).

Considering the current major data gaps, it is likely that in most cases, experimental data would be needed to substantiate and justify the use of a grouping and read-across approach for NMs.

The main barriers to the adoption of *in silico* methods for the risk assessment of NMs in cosmetics include the limited understanding of the physical interaction of NMs with biological systems, the lack of standardized, validated assays for nanosafety testing, the limited usefulness of the commonly available *in silico* models or systems in assessing NM toxicity, the development status of *in silico* modeling approaches for the prediction of biological and toxicological responses to NMs in cosmetics, and data gaps in *in vivo* behavior and effects for different groups of NMs.

4. Conclusions and outlook

The EU legislation addressing chemicals includes overarching legislation addressing chemicals in general (REACH (EC 2006) and CLP (EC 2008a)) and sector-specific legislation for specific uses of chemicals. The latter type of legislation operates with positive lists (i.e. lists of substances approved for a certain use) and, in some cases, negative lists. Compared with REACH and CLP, the number of chemicals addressed by sector-specific legislation is significantly smaller, although since either the hazard of or human exposure to such chemicals is considered higher than that of chemicals in general, sector-specific regulatory information requirements can be more thorough than those under REACH (e.g. BPR (EU 2012a) and PPPR (EC 2009a)).

Definition of 'nanomaterial' in different pieces of legislation

Table 1 gives an overview of how NMs are defined and addressed under EU chemicals legislation.

REACH (EC 2006) does not yet define NMs, but an agreement has been reached to amend the REACH annexes and include a definition of 'nanoform' (EU 2018), which is expected to take place during 2018; after this also REACH, and hence CLP, will define NMs. Regarding the sector-specific legislation, the BPR (EU 2012a) was the first legal act to define NMs on basis of the EC Definition (EC 2011); like the BPR, the Novel Foods Regulation (EU 2015b) and the Food Information to Consumers (EU 2011b) apply a definition that differs from the EC Definition in terms of the threshold for the number proportion of particles at the nanoscale. The CPR (EC 2009b) defines NMs as intentionally produced and insoluble or biopersistent, which differs from the EC definition (EC 2011). The plastic food contact materials regulation (EU 2011a) as well as the legislation for various types of additives to food (EC 2008b, 2008c, 2008d) refer either to the use of nanotechnology or to manufactured (engineered) NMs, without however giving a definition. The PPPR (EC 2009a) and the FCM regulation (EC 2004) do not explicitly mention NMs.

4.1. Possibilities for using grouping and read-across within the different pieces of legislation

Table 1 gives an overview of the possibilities for using grouping and read-across under the pieces of legislation reviewed.

REACH, together with CLP, encourages the use of alternative approaches, including grouping and

read-across. Under BPR, the provisions of Annex IV establish rules for grouping and read-across that are almost identical to those under REACH; thus in principle, grouping and read-across can be applied for assessing NMs under BPR. The PPPR does not exclude using alternative methods for the evaluation of products and, in practice, toxicity data for the required endpoints are often extrapolated from similar products, based on information on the active substance(s) and co-formulants (Kah et al. 2013). The CPR lays down the rules for human health risk assessment of substances used in cosmetic products, whereas their environmental risk assessment is performed under REACH; read-across and grouping is in principle possible, if the underlying data are sufficiently robust to enable this approach (SCCS 2013a). The FCM regulation does not exclude applying alternative methods for evaluating human health hazards, and thus it should be possible to use grouping and read-across in toxicological assessments.

In the risk assessment of substances added to food, various alternative methods are allowed for filling in data gaps for specific endpoints. For novel foods, substances are assessed case by case, and for novel foods containing engineered NMs, the scientific appropriateness of the (adaptations of) test methods used must be justified (EU 2015b); in the absence of specific guidance on grouping and read-across, these methods are rarely used for assessing NMs in novel foods. In plastic FCMs, only NMs that have been explicitly authorized and entered onto the positive list are allowed. The authorization process excludes using grouping and read-across for NMs for the time being, and therefore NMs must be individually assessed. Thus this sector-specific legislation would need to be amended to allow grouping.

4.2. Availability of guidance for applying grouping and read-across within the different pieces of legislation

Regarding REACH and CLP, guidance for grouping and read-across (ECHA 2008, 2017b), also specifically addressing NMs (ECHA 2017a, 2017c), are available. There is no specific guidance for grouping of NMs under the BPR or PPPR, and scientifically sound approaches and tools for grouping and read-across of NMs used in biocidal or plant protection products remain to be developed.

EFSA guidance (EFSA 2011; currently being updated) addressing the application of nanotechnology in the food and feed chain states that risk assessment of NMs used in FCMs may use relevant information obtained by read-across from other NMs or non-nanoforms. The guidance emphasizes the importance of adequate characterization for establishing the identity of NMs in food/feed products and under testing conditions, for example, as pristine material, and as test material in food matrices. For NMs that completely dissolve or degrade in the gastro-intestinal tract, hazard assessment can rely on data for the non-nanoform (if available), as long as the possibility of NM absorption before the dissolution or degradation stage can be excluded. The implementation of grouping and read-across under the food and novel foods legislation will depend on the availability of extensive characterization data and the development of reliable methodology for assessing individual novel foods containing engineered NMs. EFSA has proposed guidance on the toxicological assessment of NMs (CEF 2016b), which may help to implement read-across approaches. EFSA is currently working to *'clarify concept and/or principles on how nanoforms can be "clustered" into different groups*' (EFSA 2016).

For cosmetic products, guidance documents are available from the SCCS for performing risk assessment of NMs (SCCS 2012a, 2013a, 2015a, 2016a, 2017a). While there is no specific guidance for grouping, for the risk evaluation of cosmetic ingredients, including NMs, all available scientific data are considered, including that obtained through grouping and read-across.

4.3. Actual assessments performed

REACH requires that for substances placed on the market in quantities above 10 tons per year, a chemical safety assessment is performed. Any substance placed on the market must be classified and labeled according to CLP. While some NMs have been approved for biocidal use (EC 2017, 2014), their approval has been based on the evaluation of test data, not data obtained through grouping and read-across. Very few nano-PPPs have so far been evaluated under PPPR (Aschberger et al. 2015).

Under CPR, the SCCS has published scientific opinions on several NMs (SCCS 2011, 2012b, 2013b, 2013c, 2015b, 2015c, 2015d, 2017b). The assessment of the use of titanium dioxide as an UV-filter in dermal cosmetic products could be considered an example of applying read-across (SCCS 2013b, 2016b) for an ingredient in a cosmetic product. For FCMs, grouping approaches and read-across have been applied for a few non-nano substances for certain endpoints (CEF 2015a, 2015b, 2016b); all NMs have so far been evaluated case by case. Regarding substances added to food, read-across has been applied to some food additives and flavoring agents (CEF 2009), none of them NMs.

4.4. Final comments

Considering the current major data gaps, it seems likely that in most cases, experimental data are needed to substantiate and justify the use of a grouping and read-across approach for NMs.

An additional general challenge to applying grouping and read-across in assessing the safety of NMs is that different pieces of legislation define NMs differently (see Table 1). Industry must be able to accurately identify ingredients as NMs, as defined by the relevant legislation, in order to comply with nanospecific information, testing or labeling requirements (e.g., EC 2009b, EU 2012a, 2012b). Once the EC review of the EC definition (EC 2011) is finalized, a definition harmonized across legislation, as far as applicable, may be expected.

Since REACH (and CLP) cover nearly all substances, these pieces of legislation would seem to have the highest need for alternative testing approaches. They are the logical starting point for developing grouping and read-across approaches which, as relevant, can then be implemented under other legislation, unless explicitly excluded. Legislation controlling occupational exposures to chemicals (e.g., EC 1998) and major accident hazards (EU 2012b) are further areas where grouping approaches could perhaps be applied.

Another aspect of applying grouping and read-across is the (un)availability of methods for doing so. Current concepts for the grouping of NMs for human health risk assessment (e.g., Arts et al. 2014 and 2015, ECHA et al. 2016) go beyond determining structure-activity relationships and consider, e.g., morphology, surface reactivity, toxicokinetics, biological effects, and NM life cycle aspects. However, none of the approaches take all aspects fully into account, and some are limited to one exposure route only (e.g. Arts et al. 2015). Thus, different concepts need to be compared, integrated and validated, for example by applying them to different case studies. As an example, the main barriers to the adoption of *in silico* methods for grouping and read-across in the risk evaluation of NMs as cosmetic ingredients include the limited understanding of the physical interaction of NMs with biological systems, the lack of standardized, validated assays for nanosafety testing, the development status of *in silico* modeling approaches for the prediction of biological and toxicological responses to NMs in cosmetics, and data gaps in *in vivo* behavior and effects for different groups of NMs (SCCS 2012a).

The availability of sound principles for grouping will also support the introduction of Safe(r)-by-Design concepts into the design and manufacturing processes of novel NMs by facilitating the assessment of hazard-related properties at different stages of the manufacturing process (Brehm et al. 2017), and thus providing effective means to optimize the safety of the final product and to fulfill regulatory safety requirements for putting the final product on the market.

There is consensus (e.g., ECHA et al. 2016, OECD 2016b) that for NMs, particularly nanoforms of the same chemical composition, grouping and read-across can help to reduce testing while still obtaining sufficient information to assess their risks. Specific guidance for implementing grouping and read-across of NMs for (eco)toxicological and physico-chemical endpoints still needs to be developed, both within the EU and the OECD. Under REACH, ECHA has issued a guidance appendix relevant for NMs (ECHA 2017c) in addition to its guidance on the grouping for chemicals (ECHA 2008); OECD, however, concluded in its latest edition of the 'Guidance on grouping of chemicals' that '*at present, it seems premature to develop guidance on grouping specifically for nanomaterials*'. (OECD 2014).

Most importantly, criteria are needed for grouping nanoforms and distinguishing the different nanoforms of one substance within a REACH registration. Guidance is needed for identifying the most potent, 'worst case', or 'representative' nanoform

within a group. Considering the analytical challenges of the comprehensive characterization of NMs, standardized implementation instructions are needed and required also for demonstrating similarity between different nanoforms or between nanoforms and non-nanoforms of a substance. A future consideration is read-across also between nanoforms of different substances.

The existing methodologies for risk assessment seem to be appropriate for assessing the potential risks associated with NMs (SCENIHR 2006). However, for some legislation, nanospecific guidance still has to be developed. OECD also considers the current test guidelines and testing strategies in general appropriate for assessing the risk of NMs, although they may have to be adapted to the specificities of NMs. As a first step, test guidelines for inhalation toxicity have been updated for NMs (OECD 2017a, 2017b), and a new TG on dispersion stability (OECD 2017c) has also been published. Other OECD TGs are currently being updated or developed for NMs as well.

An issue specific to CPR is the ban on animal testing, which means that validated alternative test methods also need to be validated for NMs.

A consensus seems to emerge that it should be possible to develop criteria and conditions that allow the risk assessment of NMs based on a category approach rather than on a case-by-case basis. It also seems relevant to elaborate on the options for the analog approach to broaden the possibilities of grouping and read-across of NMs.

Disclosure statement

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References

Arts, J. H. E., M. Hadi, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel., et al. 2014. "A Critical Appraisal of Existing Concepts for the Grouping of Nanomaterials." *Regulatory Toxicology and Pharmacology* 70 (2): 492–506.

Arts, J. H. E., M. Hadi, M.-A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier., et al. 2015. "A Decision-Making Framework for the Grouping and Testing of Nanomaterials (DF4nanoGrouping)." *Regulatory Toxicology and Pharmacology* 71 (2): S1–S27.

Aschberger, K., S. Gottardo, V. Amenta, M. Arena, F. Botelho Moniz, H. Bouwmeester, P. Brandhoff., et al. 2015. "Nanomaterials in Food – Current and Future Applications and Regulatory Aspects." *Journal of Physics: Conference Series* 617: 012032.

Brehm, M., A. Kafka, M. Bamler, R. Kühne, G. Schüürmann, L. Sikk, J. Burk., et al. 2017. "An Integrated Data-Driven Strategy for Safe-by-Design Nanoparticles: The FP7 MODERN Project." *In Modelling the Toxicity of Nanoparticles*, edited by L. Tran, M. A. Bañares, and R. Rallo, 257–301. Cham, Switzerland: Springer International Publishing.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids] 2013. "Toxicological Evaluation of Benzophenone." *Efsa J* 11 (4): 3189.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids] 2012. "Scientific Opinion on the Safety Evaluation of the Substance, Titanium Nitride, Nanoparticles, for Use in Food Contact Materials." *Efsa J* 10 (3): 2641.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids] 2014a. "Statement on the Safety Assessment of the Substance Silicon Dioxide, Silanated, FCM Substance No 87 for Use in Food Contact Materials." *Efsa J* 12 (6): 3712.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids], 2014b. "Scientific Opinion on the Safety Assessment of the Substance (Methacrylic Acid, Ethyl Acrylate, n-Butyl Acrylate, Methyl Methacrylate and Butadiene) Copolymer in Nanoform for Use in Food Contact Materials." *Efsa J* 12 (4): 3635.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids], 2015a. "Scientific Opinion on the Safety Assessment of the Substance Ethylene Glycol Dipalmitate, CAS No 624-03-3, for Use in Food Contact Materials." *EFSA Journal* 13 (2): 4019.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids], 2015b. "Scientific Opinion on the Safety Assessment of the Substance Fatty Acids, C16-18 Saturated, Hexaesters with Dipentaerythritol for Use in Food Contact Materials." *EFSA Journal* 13 (2): 4021.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids], 2016a. "Safety Assessment of the Substance Zinc Oxide, Nanoparticles, for Use in Food Contact Materials." *EFSA Journal* 14 (3): 4408.

CEF [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids] 2016b. "Recent Developments in the Risk Assessment of Chemicals in Food and Their Potential Impact on the Safety

Assessment of Substances Used in Food Contact Materials." *Efsa J* 14 (1): 4357.

Dekkers, S., Oomen, A. G., Bleeker, E. A. J., Vandebriel, R. J., Micheletti, C., Cabellos, J., Janer, G. G., et al. 2016. "Towards a Nanospecific Approach for Risk Assessment." *Regulatory Toxicology and Pharmacology* 80: 46–59.

EC 1998. Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work. OJ No. L131, 5.5.1998, p. 11.

EC 2002. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements OJ L 183, 12.7.2002, p. 51

EC 2004. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ No. L 338, 13.11.2004, p. 4.

EC 2005. Communication from the Commission to the Council, the European Parliament and the Economic and Social Committee. Nanosciences and nanotechnologies: An action plan for Europe 2005-2009. COM(2005) 243 final, Brussels, Belgium. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52005DC0243.

EC 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ No. L396, 30.12.2006, p.1.

EC 2008a. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (CLP). OJ No. L353, 31.12.2008, p. 1

EC 2008b. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ No. L354, 31.12.2008, p. 16

EC 2008c. Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes. OJ No. L354, 31.12.2008, p. 7.

EC 2008d. Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. OJ No. L354, 31.12.2008, p. 34.

EC 2009a. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market. OJ No. L309, 24.11.2009, p. 1.

EC 2009b. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ No. L342, 22.12.2009, p. 59

EC 2009c. Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. OJ L 135, 30.5.2009, p. 3.

EC 2011. Commission recommendation of 18 October 2011on the definition of nanomaterial. 2011/696/EU. OJ No. L 275, 20.10.2011, p. 38.

EC 2014. Commission Implementing Regulation (EU) No 408/2014 of 23 April 2014 approving synthetic amorphous silicon dioxide as an existing active substance for use in biocidal products for product-type 18. OJ No. L121, 24.4.2014, p. 17.

EC 2017. Commission Implementing Regulation (EU) 2017/795 of 10 May 2017 approving pyrogenic, synthetic amorphous, nano, surface treated silicon dioxide as an existing active substance for use in biocidal products of product-type 18. OJ No. L120, 11.5.2017, p. 10–12.

EC 2018. Commission Regulation (EU) … / … of XXX amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III,VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances, and ANNEX. 1. Document D056122/02 of 26 April 2018. Available at http://ec.europa.eu/transparency/regcomitology/index.cfm? do=search.documentdetail&Dos_ID =15915&DS_ID =56122&Version =2

ECHA 2008. *Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals;* Helsinki: ECHA. Available at: https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf

ECHA 2012a. Background Paper: An introduction to the assessment of read-across in ECHA; Expert workshop on read-across assessment with the active support of Cefic LRI, 2–3 October 2012. Helsinki, ECHA. Available from https://echa.europa.eu/documents/10162/22816103/ws_raa_20121003_background_paper_an_introduction_to_the_assessment_of_read-across_in_echa_en.pdf.

ECHA 2012b. Guidance on information requirements and chemical safety assessment, Appendix R8-15 Recommendations for nanomaterials applicable to Chapter R.8 Characterisation of dose [concentration] - response for human health. Helsinki: ECHA, ECHA-12-G-09-EN. Available at: https://echa.europa.eu/documents/10162/23047722/appendix_to_r8_clean_en.pdf/49741897-f351-4180-a841-3b1e6dde185a

ECHA 2012c. Guidance on information requirements and chemical safety assessment, Appendix R10-2 Recommendations for nanomaterials applicable to Chapter R.10 Characterisation of dose [concentration] - response for environment. Helsinki: ECHA, ECHA-12-G-10-EN. Available at: https://echa.europa.eu/documents/10162/23047722/appendix_to_r10_clean_en.pdf/4da90e08-4674-424c-a6b7-b3abdb97691c

ECHA 2012d. Guidance on information requirements and chemical safety assessment, Appendix R14-4 Recommendations for nanomaterials applicable to Chapter R.14 Occupational exposure estimation. Helsinki: ECHA, ECHA-12-G-08-EN. Available at: https://echa.europa.eu/documents/10162/23047722/appendix_to_r14_clean_en.pdf/20629d45-2c0a-4038-aa51-77d82e22342f

ECHA 2013a. *Grouping of Substances and Read-Across Approach, Part 1: Introductory Note*; Helsinki: ECHA, ECHA-13-R-02-EN. Available at: https://echa.europa.eu/documents/10162/13628/read_across_introductory_note_en.pdf

ECHA 2013b. *Assessing human health and environmental hazards of nanomaterials – Best practice for REACH Registrants; Second GAARN meeting, Helsinki, 21–22 Jan. 2013*. Helsinki: ECHA, ECHA-13-R-04-EN. Available at: https://echa.europa.eu/documents/10162/5399565/best_practices_human_health_environment_nano_en.pdf/8e0adb6a-829c-43aa-84c5-5361f8505996

ECHA 2014a. Human health and environmental exposure assessment and risk characterization of nanomaterials – Best practice for REACH registrants. Third GAARN Meeting, Helsinki, 30 September 2013; Helsinki: ECHA, ECHA-14-R-10-EN. Available at: https://echa.europa.eu/documents/10162/5399565/best_practices_human_health_environment_nano_3rd_en.pdf/a368f6df-fe40-4673-a041-83b63cc9f50b

ECHA 2014b. *Opinion on a request according to Article 75(1)(g) of Regulation (EU) No 528/2012, HeiQ AGS-20*. Helsinki: ECHA, ECHA/BPC/001/2014. Available at: https://echa.europa.eu/documents/10162/21680461/bpc_opinion_heiq_ags-20_en.pdf

ECHA 2016a. Topical Scientific Workshop on New Approach Methodologies in Regulatory Science, Helsinki, 19–20 April 2016, Background Document; Helsinki: ECHA. Available at: https://echa.europa.eu/documents/10162/22816069/tsws_background_document_en.pdf/53102457-43f9-4709-8e4f-cf2b58458fbf

ECHA 2016b. *Report on the Operation of REACH and CLP 2016*. Helsinki: ECHA, ECHA-16-R-08-EN. Available at: https://www.echa.europa.eu/documents/10162/13634/operation_reach_clp_2016_en.pdf

ECHA et al. 2016. Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance. Elements to consider; Helsinki: ECHA, ED-02-16-228-EN-N. Available at: https://echa.europa.eu/documents/10162/13630/eco_toxicological_for_bridging_grouping_nanoforms_en.pdf

ECHA 2017a. *How to prepare registration dossiers that cover nanoforms: best practices*. Helsinki: ECHA, ECHA-17-G-13-EN. Available at: https://www.echa.europa.eu/documents/10162/13655/how_to_register_nano_en.pdf/f8c046ec-f60b-4349-492b-e915fd9e3ca0

ECHA 2017b. *Read-Across Assessment Framework (RAAF)*; Helsinki: ECHA, ECHA-17-R-01-EN. Available at: https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

ECHA 2017c. Guidance on information requirements and chemical safety assessment, Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals. Helsinki: ECHA, ECHA-17-G-17-EN. Available at: https://www.echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf/71ad76f0-ab4c-fb04-acba-074cf045eaaa

ECHA 2017d. Guidance on information requirements and chemical safety assessment, Appendix R7-1 for nanomaterials applicable to Chapter R7a Endpoint specific guidance. Helsinki: ECHA, ECHA-17-G-16-EN. Available at: https://echa.europa.eu/documents/10162/22334053/draft_appendix_r7a_msc_en.pdf/c2b2e297-86b2-a1ee-f02a-72938c83d362

ECHA 2017e. Guidance on information requirements and chemical safety assessment, Appendix R7-1 for nanomaterials applicable to Chapter R7b Endpoint specific guidance. Helsinki: ECHA, ECHA-17-G-15-EN. Available at: https://echa.europa.eu/documents/10162/13632/appendix_r7b_nanomaterials_en.pdf/6eca425a-ede1-4c9e-8151-af77973caf32

ECHA 2017f. Guidance on information requirements and chemical safety assessment, Appendix R7-2 for nanomaterials applicable to Chapter R7c Endpoint specific guidance. Helsinki: ECHA, ECHA-17-G-14-EN. Available at: https://echa.europa.eu/documents/10162/13632/appendix_r7c_nanomaterials_en.pdf/c2d8e0ce-2ab4-4035-a05f-e2dec924d87a

ECHA 2017gg., *Functionalities of IUCLID 6*; Helsinki: ECHA. Available at: https://iuclid6.echa.europa.eu/documents/21812392/22308501/iuclid_functionalities_en.pdf/559b80ad-cef7-4edb-8c2b-ca52e0d511e2

ECHA 2017hh., Guidance on the Application of the CLP Criteria – Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Helsinki: ECHA, ECHA-17-G-21-EN. https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5

EFSA, 2011. "Guidance on the Risk Assessment of the Application of Nanoscience and Nanotechnologies in the Food and Feed Chain." *Efsa J* 9 (5): 2140.

EFSA 2016. "*Annual Report of the EFSA Scientific Network of Risk Assessment of Nanotechnologies in Food and Feed for 2015*; Parma: EFSA, 2016." *EFSA Supporting Publications* 13 (1): EN-939.

EU 2011a. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ No. L12, 15.1.2011, p. 1.

EU 2011b. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. OJ No. L304, 22.11.2011, p. 18.

EU 2012a. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167, 27.6.2012, p. 1.

EU 2012b. Directive 2012/18/EU of the European Parliament and of the Council of 4 July 2012 on the control of major-accident hazards involving dangerous substances. OJ L 197, 24.7.2012, p. 1.

EU 2013. Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control OJ L 181, 29.6.2013, p. 35.

EU 2015a. Commission Regulation (EU) 2015/174 of 5 February 2015 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. OJ No. L30, 6.2.2015, p. 2.

EU 2015b. Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011. OJ No. L327, 11.12.2015, p. 1.

EU 2016. Commission Regulation (EU) 2016/1416 of 24 August 2016 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. OJ No. L230, 25.8.2016, p. 22.

Grafström, R. C., P. Nymark, V. Hongisto, O. Spjuth, R. Ceder, E. Willighagen, B. Hardy, S. Kaski, and P. Kohonen. 2015. "Toward the Replacement of Animal Experiments through the Bioinformatics-Driven Analysis of 'Omics' Data from Human Cell Cultures." *Alternatives to Lab Animals* 43 (5): 325–32.

Hund-Rinke, K., C. Nickel, and D. Kühnel. 2017. Considerations about the relationship of nanomaterial's physical-chemical properties and aquatic toxicity for the purpose of grouping. Report No. TEXTE 102/2017. (UBA-FB) 002572/ENG. Available from: https://www.umweltbundesamt.de/publikationen/considerations-about-the-relationship-of.

Kah, M., S. Beulke, K. Tiede, and T. Hofmann. 2013. "Nanopesticides: state of Knowledge, Environmental Fate and Exposure Modelling." *Critical Reviews in Environmental Science and Technology* 43 (16): 1823–67.

Kleinstreuer, N. C., K. Sullivan, D. Allen, S. Edwards, D. L. Mendrick, M. Embry, J. Matheson., et al. 2016. "Adverse Outcome Pathways: From Research to Regulation Scientific Workshop Report." *Regulatory Toxicology and Pharmacology* 76: 39–50.

OECD 2009. Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials. Paris: OECD, ENV/JM/MONO(2009)21. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2009)21

OECD 2014. *Guidance on Grouping of Chemicals, Second Edition*. Paris: OECD, ENV/JM/MONO(2014)4. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage = en

OECD 2016a. *Categorisation of Manufactured Nanomaterials – Workshop Report*. Paris: OECD, ENV/JM/MONO(2016)9. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282016%299&doclanguage=en

OECD 2016b. *Grouping and Read-across for the Hazard Assessment of Manufactured Nanomaterials, Report from the Expert Meeting*. Paris: OECD, ENV/JM/MONO(2016)59. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)59&doclanguage=en

OECD 2016c. Approaches on Nano Grouping/Equivalence/Read-across Concepts based on Physical-chemical Properties (GERA-PC) for Regulatory Regimes, Results from the Survey. Paris: OECD, ENV/JM/MONO(2016)3. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2016)3&docLanguage=En

OECD 2017a. *Subacute Inhalation Toxicity: 28-Day Study; OECD Guidelines for the Testing of Chemicals, Test No. 412*. Paris: OECD. Available at: https://www.oecd-ilibrary.org/environment/test-no-412-subacute-inhalation-toxicity-28-day-study_9789264070783-en

OECD 2017b. *Subchronic Inhalation Toxicity: 90-day Study; OECD Guidelines for the Testing of Chemicals, Test No. 413*. Paris: OECD. Available at: https://www.oecd-ilibrary.org/environment/test-no-413-subchronic-inhalation-toxicity-90-day-study_9789264070806-en

OECD 2017c. Dispersion Stability of Nanomaterials in Simulated Environmental Media; OECD Guidelines for the Testing of Chemicals, Test No. 318. Paris: OECD. Available at: http://www.oecd.org/env/ehs/testing/test-no-318-dispersion-stability-of-nanomaterials-in-simulated-environmental-media-9789264284142-en.htm

Oomen, A. O., E. A. J. Bleeker, P. M. J. Bos, F. van Broekhuizen, S. Gottardo, M. Groenewold, D. Hristozov., et al. 2015. "Grouping and Read-across Approaches for Risk Assessment of Nanomaterials." *International Journal of Environmental Research and Public Health* 12 (10): 13415–34.

Rasmussen, K., H. Rauscher, A. Mech, J. Riego Sintes, D. Gilliland, M. González, P. Kearns., et al. 2018. "Physico-Chemical Properties of Manufactured Nanomaterials – Characterisation and Relevant Methods. An Outlook Based on the OECD Testing Programme." *Regulatory Toxicology and Pharmacology* 92: 8–28.

SCCS 2011. *Opinion on nano-ETH50 (1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-) as a UV filter in cosmetic products*. European Union: SCCS, SCCS/1429/11. Available at: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_070.pdf

SCCS 2012a. *Guidance on the safety assessment of nanomaterials in cosmetics*. European Union: SCCS, SCCS/1484/12. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_005.pdf

SCCS 2012b. *Opinion on zinc oxide (nano form) COLIPA S 76*. European Union: SCCS, SCCS/1489/12. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_103.pdf

SCCS 2013a. *Memorandum on "Relevance and Quality of Data in Safety Dossiers on Nanomaterials"*. European Union: SCCS, SCCS/1524/13. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_142.pdf

SCCS 2013b. *Opinion on titanium dioxide (nano form) COLIPA n° S75*. European Union: SCCS, SCCS/1516/13. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_136.pdf

SCCS 2013c. *Opinion on Carbon Black (nano-form)*. European Union: SCCS, SCCS/1515/13. Available at: http://ec.europa.

eu/health/scientific_committees/consumer_safety/docs/sccs_o_144.pdf

SCCS 2015a. *The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 9th revision*. European Union: SCCS, SCCS/1564/15. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf

SCCS 2015b. *Opinion on Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form)*. European Union: SCCS, SCCS/1545/15. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_175.pdf

SCCS 2015c. Opinion on 2,2′-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) (nano form), Submission III, COLIPA n° S79. European Union: SCCS, SCCS/1546/15. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_168.pdf

SCCS 2015d. *Opinion on Hydroxyapatite (nano)*. European Union: SCCS, SCCS/1566/15. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_191.pdf

SCCS 2016a. *Memorandum on the Use of In Silico Methods for Assessment of Chemical Hazard*. European Union: SCCS, SCCS/1578/16. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_200.pdf

SCCS 2016b. *Opinion on additional coatings for Titanium Dioxide (nano form) as UV-filter in dermally applied cosmetic products*. European Union: SCCS, SCCS/1580/16. Available at: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_202.pdf

SCCS 2017a. *Checklists for Applicants submitting dossiers on Cosmetic Ingredients to be evaluated by the SCCS*. European Union: SCCS, SCCS/1588/17. Available at: https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_210.pdf

SCCS 2017b. *Opinion on Titanium Dioxide (nano form) as UV-Filter in sprays*. European Union: SCCS, SCCS/1583/17. Available at: https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_206.pdf

SCENIHR 2006. Modified Opinion (after public consultation) on The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies. European Union: SCENIHR, SCENIHR/002/05. Available from: https://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003b.pdf.

Sellers, K., N. M. E. Deleebeeck, M. Messiaen, M. Jackson, E. A. J. Bleeker, D. T. H. M. Sijm, and F. A. van Broekhuizen. 2015. Grouping nanomaterials. A strategy towards grouping and read-across. RIVM Report 2015-0061. ISBN 978-90-6960-280-6. Available from: http://www.rivm.nl/bibliotheek/rapporten/2015-0061.html.

Teixeira do Amaral, R., J. Ansell, N. Aptula, T. Ashikaga, Q. Chaudhry, A. Hirose, J. Jaworska., et al. 2014. *In Silico Approaches for Safety Assessment of Cosmetic Ingredients, Report for the International Cooperation on Cosmetics Regulation*. International Cooperation on Cosmetics Regulation. Available from: www.iccr-cosmetics.org/files/5314/1407/7607/2014-07_In-silico_Approaches_for_Cosmetic_Product_Safety_Assessments.pdf