Nanotoxicology

ISSN: 1743-5390 (Print) 1743-5404 (Online) Journal homepage: https://www.tandfonline.com/loi/inan20

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To cite this article: L. Lamon, K. Aschberger, D. Asturiol, A. Richarz & A. Worth (2019) Grouping of nanomaterials to read-across hazard endpoints: a review, Nanotoxicology, 13:1, 100-118, DOI: 10.1080/17435390.2018.1506060

To link to this article: https://doi.org/10.1080/17435390.2018.1506060

NANOTOXICOLOGY
2019, VOL. 13, NO. 1, 100–118
https://doi.org/10.1080/17435390.2018.1506060

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Grouping of nanomaterials to read-across hazard endpoints: a review

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ABSTRACT

The use of non-testing strategies like read-across in the hazard assessment of chemicals and nanomaterials (NMs) is deemed essential to perform the safety assessment of all NMs in due time and at lower costs. The identification of physicochemical (PC) properties affecting the hazard potential of NMs is crucial, as it could enable to predict impacts from similar NMs and outcomes of similar assays, reducing the need for experimental (and in particular animal) testing. This manuscript presents a review of approaches and available case studies on the grouping of NMs to read-across hazard endpoints. We include in this review grouping frameworks aimed at identifying hazard classes depending on PC properties, hazard classification modules in control banding (CB) approaches, and computational methods that can be used for grouping for read-across. The existing frameworks and case studies are systematically reported. Relevant nanospecific PC properties taken into account in the reviewed frameworks to support grouping are shape and surface properties (surface chemistry or reactivity) and hazard classes are identified on the basis of biopersistence, morphology, reactivity, and solubility.

ARTICLE HISTORY
Received 3 January 2018
Revised 25 June 2018
Accepted 23 July 2018

KEYWORDS
Grouping; nanomaterial; read-across; hazard assessment; REACH regulation

Introduction

The risk assessment of chemicals is traditionally based on toxicity studies on animals, which serve as surrogates for humans. With growing concerns about animal welfare and the questionable relevance of animal tests, legal requirements, and technological advances, new possibilities to determine the hazardous properties of substances that do not require the use of animals are increasingly available. The application of alternative (non-animal) methods is supported by the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation (European Parliament and Council 2006) as exploratory (e.g. to better understand mechanisms of action) or predictive tools (to extrapolate observations to the whole organism level) in hazard assessment. Read-across is based on the assumption that chemicals that are considered similar, based on their chemical composition and/or physicochemical (PC) properties, may have comparable toxicokinetic and toxicodynamic properties. Therefore, experimental available toxicological properties from a 'source' chemical can be used to derive toxicological properties of a (structurally similar) 'target' analogue with no (or limited) toxicological experimental data: thus, the unknown toxic effects of a chemical of interest can be predicted from the known effects of one or more analogues.

The risk assessment of engineered nanomaterials (NMs) is implicitly addressed by REACH (Wahid et al. 2017) and in principle the development of groups or categories of NMs should provide a valuable means of filling data gaps for relevant hazard endpoints of NMs. Several approaches to read-across NMs have been proposed in the literature (Oomen et al. 2018). It should be noted that the terminology is not used consistently in different sources, so that 'group' or 'category' do not always correspond with the use of this term under REACH (European Parliament and Council 2006), where it is defined as follows:

> Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural

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> similarity may be considered as a group, or "category" of substances. Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach).[1]

This principle has been recently reaffirmed by the European Chemicals Agency (ECHA), releasing the guidance for REACH registrants on how to justify the use of hazard data between nanoforms of the same substance (ECHA 2017). Grouping approaches applicable to NMs were under discussion before the release of guidance from ECHA, and before any adoption of grouping guidance by authorities as thoroughly discussed by Arts et al. (2014).

Various schemes for grouping of NMs have been proposed covering a variety of assessment goals, including: (a) priority setting of NMs for further evaluation (including ranking based on level of concern) (e.g. Nel et al. 2013; Cockburn et al. 2012), (b) guiding the choice of relevant endpoints and methods in testing strategies (Godwin et al. 2015; Hansen et al. 2007; Stone et al. 2014, 2013), and (c) grouping and read-across for the purpose of filling data gaps in regulatory submissions for hazard endpoints (e.g. Sellers et al. 2015), which is the application of particular interest in this review article.

The objective of this manuscript is to report the state-of-the-art in grouping approaches for the hazard assessment of NMs, including case studies when available. The selection reported in this review focuses on hazard-based approaches. In this article our aims are to: (1) classify the different existing approaches with respect to REACH application considering grouping for read-across, and (2) identify PC properties that are relevant in the different approaches. Furthermore, according to available approaches and related case studies, we will identify the research needs and provide recommendations on the next steps to improve read-across of NMs for hazard endpoints.

Methods

We have reviewed all published categorisation schemes and grouping for read-across approaches from a literature search performed in Scopus on March the 20th (2018) with the keywords Nanopart* OR Nanomat* AND Grouping OR Read-across OR Categorization* OR Prioritization* OR Ranking. Of the 457 papers resulting from the search, 34 were identified as relevant for categorisation and grouping approaches based on the abstract contents and on were considered reliable according to the analysis reported in the main text. These papers are either cited in the text or reported thoroughly in Tables 1–3. As our interest is to report hazard-based grouping, we have included in Table 2 also approaches reported in Liguori et al. (2016). Other reviews are available that report on existing risk assessment frameworks (e.g. Oomen et al. 2018) or on *in silico* tools applied to NMs (e.g. Chen et al. 2018). In this manuscript, we have made a selection of approaches that are relevant for developing an acceptable grouping hypothesis based on experimental evidence.

Grouping approaches and frameworks applied to nanomaterial hazard assessment

ECHA released a guidance document on information requirements and chemical safety assessment specific to the application of QSARs and grouping approaches to NMs (ECHA 2017), and on how to justify grouping for read-across between nanoforms of the same substance. This guidance has taken into account some key concepts and considerations related to NM grouping and read-across identified by the ECHA Group Assessing Already Registered Nanomaterials (GAARN) and the ECHA Nanomaterials Working Group (NMWG) as well as a strategy presented earlier (RIVM, JRC, and ECHA, 2016). It includes the need to consider properties beyond chemical composition (e.g. aspect ratio, particle size, shape, or solubility), the reaffirmation of the similarity rules from REACH Annex XI for NMs (European Parliament and Council 2006), the relevance of toxicokinetic studies (and toxicokinetic proxies), in grouping, read-across, and for *in vitro* to *in vivo* extrapolation (ECHA 2013a, 2013b).

Likewise the OECD (2014) has acknowledged the need to develop frameworks for grouping of NMs. The European Food and Safety Authority (EFSA) identified the relevance for read-across in risk assessment of NMs (EFSA Scientific Committee 2011). Guidance on how to consider and integrate

Table 1. List of frameworks proposed for NM grouping.

Approach (reference)	Assessment goal(s)	Basis for grouping	Predefined groups	Testing strategy supported	Practicality (standard methods identified)	Applicability	Comments on applicability within REACH
DF4nanoGrouping (Arts et al. 2015)	Human health hazard assessment (inhalation exposure)	Assignment to one of the four pre-defined categories is based on • water solubility, • particle morphology and composition, • dissolution rate, • surface reactivity, • dispersability, • *in vitro* reactivity Confirmation of the categorisation of a NM comes through information from *in vivo* studies (biopersistence, biodistribution, and genotoxicity). Use release and exposure information are applied as qualifiers to support grouping or appropriate testing	Four categories are identified: • Soluble NMs • biopersistent high aspect ratio NMs • Passive NMs • Active NMs	Exposure-based test waiving is supported; toxicological tests are identified for the inhalation route	Preferred methods, protocol, and existing test guidelines are identified (for measurement of PC properties)	The approach is applied to carbonaceous, metal sulphate NMs, metal oxides, amorphous silica, and pigments. Belonging of a NM to a pre-defined class determined testing strategy and risk management	The approach takes into account REACH requirements, and aims at test waiving. The proposed testing strategy focuses on inhalation route of exposure (oral and dermal exposure are briefly addressed)
MARINA framework (Oomen et al. 2015)	Human health and environmental hazard and risk assessment	A group includes NMs with low variability in PC, exposure, (eco)-toxicological kinetic, or fate properties	Suggested predefined categories: • quickly dissolving NMs • passive NMs • active NMs • NMs with high aspect ratio	Yes: the Marina Risk Assessment Strategy is supported	Lack of standard methods	No case studies presented	Generic framework suitable for application within REACH
RIVM grouping approach (Sellers et al. 2015)	Human health and environmental hazard and risk assessment	Similarity may be supported by information about • chemical identity, • particle characteristics, • fundamental transport and behavior, • activity and reactivity (the so called Tier 0 testing) No threshold to determine similarity has been developed	No	Yes, read-across is considered for each endpoint to waive tests on animals	Standard methods are mostly not available	The approach is applied to TiO_2 and Ag NMs	Read-across is supported when PC properties of the NM under evaluation are similar to the ones reported in the reference studies (then test waiving is proposed)
US-Canada Regulatory		NMs are classified on their chemical	Seven classes are identified:	Yes. a flowchart is provided where,	Lack of standard methods	No case studies	A testing strategy is identified according to

(*continued*)

Table 1. Continued.

Approach (reference)	Assessment goal(s)	Basis for grouping	Predefined groups	Testing strategy supported	Practicality (standard methods identified)	Applicability	Comments on applicability within REACH
Cooperation Council (RCC, 2013a, 2013b)	Human health and environmental safety	composition in order to identify (dis)similarities with bulk chemicals and for supporting future read-across. PC properties for each group are identified to support grouping and read-across: • size • shape • surface chemistry • solubility • composition • crystal structure	• CNTs • inorganic carbon • metal and metalloid oxides • metals, metal salts, and metalloids; • semiconductor quantum dots • organics • other classes	according to available information on exposure and PC properties, testing is suggested			the exposure route PC properties for the definition of similarity for read-across are identified for each class
US National Institute for Occupational Safety and Health (NIOSH) (Kuempel et al. 2012)	Human health risk assessment in occupational settings (inhalation)	• Particle size • shape • density • surface area • reactivity • solubility	Four categories are identified: • higher solubility particles that can reach systemic tissues • poorly soluble particles for which toxicity is related to the SSA • poorly soluble toxic NMs where reactive particle specific surface area dose determines toxicity • fibrous particles for which the toxicity is related to biopersistence and genotoxicity	A testing strategy is supported but not proposed	Specific standard methods are not identified	The approach is applied to fine and ultrafine particles (diesel exhaust particulate, carbon black in the ultrafine range). One NM falling in a predefined class would be compared to the identified reference materials and the risk estimate would be made according to identified PC properties	Occupational inhalation exposure is considered; the approach may support the selection of PC properties for hazard grouping

Table 2. List of occupational banding tools containing a hazard module (mainly from Liguori et al. 2016).

Approach (reference)	Assessment goal(s)	PC properties considered for hazard classification[a]	Hazard classes	Availability of a case study
Quantitative clustering framework (Drew et al. 2017)	Estimation of potency-based groups for pulmonary inflammation	Density, surface area, and diameter were most predictive of the potency group	Group 1–4 with one corresponding to highest potency	The framework is developed from a dataset of 18 particles (NMs and bulk) and 25 rodent studies; four potency groups are identified according to dose-response. The model is then tested on a separate dataset of six materials, using only the physicochemical information to predict the hazard potency group
ENM safety Classifier (Fortino and Grevo, 2017)	Group NMs according to toxicity	Intrinsic properties (nanospecific properties are not mentioned)	Three classes are identified as low, medium, and high toxicity	The tool is developed for 31 NMs starting from the NANOSOLUTIONS dataset and it is validated with data from MARINA project
(Oosterwijk et al. 2016)	Risk banding framework (occupational settings)	Size, net charge, hydrophobicity, solubility (at controlled pH), and ion toxicity, reactivity	Four hazard classes defined according to local and systemic toxicity, depending on net charge, solubility and reactivity and on net charge, size and hydrophobicity respectively	Conceptual framework
NanoSafer (Jensen et al. 2014)	Occupational safety (e.g. at SMEs); precautionary risk assessment	PC properties: size, shape, water solubility, and surface coating. Materials OEL; risk	Four control banding classes are identified based on toxicity	No case study available
Swiss precautionary matrix (Höck et al. 2013)	Employers and employees prioritize health risks and implement control measures	Stability, redox activity, catalytic activity, and ROS formation potential; induction potential for inflammation	Three classes of potential effects (low medium and high)	No case study available
Stoffenmanager (Van Duuren-Stuurman et al. 2012)	Human health risk assessment in occupational settings (inhalation)	Shape (fibre length). Inhalation hazard; water solubility; biopersistence	Five classes are identified (lowest to highest hazard levels are identified)	Iron powder. Falling in a risk band identifies the level of hazard control
IVAM[b] guidance (Cornelissen et al. 2011)	Workers and occupational exposure	Shape (fibrous particle) and water solubility	Three hazard categories: (water) soluble nanoparticles, synthetic, persistent nanomaterials (non-fibrous) fibrous, nonsoluble nanomaterials	NA
ANSES CB tool (ANSES, 2010)	Exposure prevention; for small to large enterprises	Reactivity, solubility (in water or in lung lining fluid) rate, shape, and biopersistence. Preliminary hazard band of the bulk material or of an analogue material: if available, the hazard band for the NM addressed is derived from that	Five hazard bands are identified (from very low to very high)	No case study available in the guidance
CB Nanotool (Zalk et al. 2009)	Oriented for nanotechnology researchers risk assessment and management	Chemical form, size, shape, surface reactivity, solubility; Information on parent material or NM: Toxicity (lowest OEL), LD50, mutagenicity, carcinogenicity, reproductive toxicology, dermal toxicity, and asthmagenicity	Four bands for identified as severity scores (taking into account both exposure and hazard information by summing the identified factors)	The tool was applied to several activities and NMs (Paik Zalk, and Swuste, 2008)

[a]Toxicity data, if used.
[b]IVAM: environmental research agency and consultancy which had its origins in the University of Amsterdam's Environmental Science Department.
ROS: reactive oxygen species; SME: small and medium enterprises; OEL: occupational exposure limit; LD50: lethal dose killing the 50% of sample.

weight of evidence in scientific assessments has been recently released by EFSA, also taking into account read-across (Hardy et al. 2017).

Current concepts, approaches, and tools for grouping of NMs have been reviewed by Oomen et al. (2018), Arts et al. (2014) and in a report by the Dutch National Institute for Public Health and The Environment (RIVM) (Sellers et al. 2015).

The proposals for hazard- and risk-based grouping approaches that propose a framework are presented in Table 1. Documents that merely provide or reiterate principles for grouping and that focus on the testing of NMs and make reference to grouping approaches, identifying some key aspects a grouping approach should focus on, but do not propose a strategy or a framework, are not listed in

Table 3. Grouping for read-across: case studies identified in the literature and computational applications that aim at ranking NMs.

Approach	Objective of the study	Methods	Dataset	Properties for grouping	Result	Comments
(Lamon et al. 2018)	Read-across hazard endpoints to fill data gaps for *in vitro* genotoxicity (comet assay)	Chemoinformatic tools such as hierarchical clustering, principal component analysis, and decision forest are applied to a dataset of TiO_2 NM to predict the outcome of the *in vitro* comet assay	Six source nanomaterials (TiO_2); read-across is made to two target TiO_2 nanoforms	Properties considered in the analysis are: • total non-TiO_2 content including coating and impurities • surface coating • organic matter • crystal type and size • shape • primary particle diameter • specific surface area • isoelectric point • density • pore volume • dustiness • biodurability • redox potential	Presence of coating was predicted to affect the outcome of the *in vitro* comet assay	This study represents an application of read-across to an *in vitro* endpoint and is presented following the ECHA proposed framework (ECHA 2017)
Sizochenko et al. (2018)	Read-across of NMs to fill data gaps for *in vitro* cytotoxicity (EC50/IC50) for bacteria, algae, protozoa, human keratinocyte cell, Balb/c 3T3	Generation of a self-organising map model is combined with interspecies correlation and is applied for data mining of the nanoparticle toxicity library to identify groups based on toxicity	30 silica and metal oxides	Enthalpy of cation formation	Four groups are identified (group 1–3 of increasing toxicity, and one corresponding to unknown toxicity)	The endpoint is not relevant for REACH. the model does not predict all the NMs taken into consideration (group 4 has unclear toxicity value)
Gajewicz et al. (2015)	Read-across in dataset of NMs to fill data gaps for the cytotoxicity endpoint (reduction of cells viability, EC50) for E. coli and HaCaT cell line (human keratinocytes)	One calculated molecular descriptor is identified in each case study (endpoint) and is used to group NMs in the Euclidean space; NMs from the validation set are (qualitatively) predicted according to the Euclidean distance	Two datasets with 17 and 18 metal oxide NMs	Euclidean distance is the similarity metric; properties considered are: • enthalpy of formation of a gaseous cation having the same oxidation state as the one in the metal oxide structure (ΔH_{Me+}) • Mulliken's electronegativity (χ^C)	Three groups of NMs with increasing toxicity properties were identified in both case studies	This study represents an application of a qualitative read-across that could be relevant according to REACH Annex XI but the endpoint is not required by REACH
Liu et al. (2015)	Consider dosimetry modeling in *in vitro* toxicity ranking	Hazard ranking was based on the EC50 and slope of the dose-response curves. Sedimentation of NMs was calculated via a fate model considering Brownian motion and gravitational settling	Seven metal oxide NMs	Hazard ranking considering delivered dose was based on dose-response analyses and compared with administered dose ranking	The comparative ranking between administered and delivered dose did not show any difference	The approach would be useful if there was an validation to *in vivo* studies
Chen et al. (2014)	Prediction of ADME by biological surface adsorption index(BSAI)	Calculation of the adsorption coefficient k as a function of five variables (describing molecular interactions); principal component analysis (PCA) for clustering	23 NMs (metal oxides, Ag, and organic NMs)	Clustering using the five identified variables	The prediction of the adsorption index was improved compared to the previous model	The approach could be applied by using the five variables as the basis for ranking and read-across to predict ADME
Zhang et al. (2012)	Validate hazard ranking based on high-throughput screening	Regression tree analysis for the effect of conduction energy band and dissolution	24 metal oxides	Cellular toxic NMs were identified depending on band gap. Dissolution was	Dissolution and conduction energy band could predict the toxicity of 7 out of 8	This application identifies a group of non-toxic NMs and two groups of toxic NMs

(*continued*)

Table 3. Continued.

Approach	Objective of the study	Methods	Dataset	Properties for grouping	Result	Comments
	(HTS) output and on *in vivo* tests	on metal oxides NMs toxicity (cell viability)		then addressed to assess the toxicological impact of NMs	NMs that were predicted (and tested) as toxic and 1 out of 8 NMs that was predicted (and tested) as non-toxic	depending on dissolution and conduction energy band. The authors show correlation between *in vitro* results and *in vivo* acute pulmonary inflammation
George et al. (2011)	Hazard ranking of a set of metal oxide NMs	Information on NM type, dose, duration of exposure, cellular targets, and cytotoxicity events was extracted from HTS data and visualised in self-organising maps and 4 groups of NMs were identified according to the observed effect	Two cell lines and four cytotoxicity responses for seven metal oxide NMs	NMs were ranked according to • similarity between their lethal response outcome or • the cytotoxic response profile of each cell line (HTS data)	NMs were ranked in four groups according to the cytotoxicity endpoints. They were ranked in five groups according to the *in vivo* tests	Hazard-based ranking of NMs. A more extensive dataset on PC properties may help to identify a group of similar NMs (structural similarity)

ADME: absorption, distribution, metabolism, and excretion; ICPMS: inductively coupled plasma mass spectrometry; SEM: scanning electron microscope; TEM: transmission electron microscope

Table 1, but are only cited. The aim of Table 1 is to report systematically the existing frameworks for grouping that are captured in the column 'Approach,' in order to easily compare the different approaches.

The FP7 project ITS Nano suggested that any approach adopted for grouping should take into account the changes occurring during the lifecycle (LC) of NMs (Stone et al. 2013). Key aspects are PC characteristics of NMs (chemical composition, size, specific surface area [SSA], etc.), their behaviour and effects (reactive oxygen species [ROS] generation, electron transfer, photoreactivity, etc.), and their fate (e.g. hydrophobicity, agglomeration, and zeta potential). The FP7 research project MARINA added to this that grouping could additionally be supported by information on kinetics (uptake, distribution, and biopersistence) and early and apical biological effects (Oomen et al. 2014). The NANOSOLUTIONS consortium goes a step forward in proposing a toxicity classifier to categorise NMs according to their toxicity, as presented in the next section (NANOSOLUTIONS 2017).

The US-Canada Regulatory Cooperation Council (RCC) has developed an approach based on chemical composition. In this approach, seven classes of NMs are defined: carbon nanotubes (CNTs); inorganic carbon; metal and metalloid oxides; metals, metal salts, and metalloids; semiconductor quantum dots; organics and other classes. In addition, toxicologically relevant PC properties are identified for each of the classes to support (sub-)classification according to the likelihood of exposure to NMs and availability of toxicity tests (RCC 2013a, 2013b). RCC (RCC 2013b) defines a flowchart to identify different groups of NMs according to solubility, biopersistence, morphology and address oral, inhalation, and dermal exposure.

The German Environment Agency (UBA, BfR, and BAuA, 2011) has recommended that grouping be based on PC properties like primary particle size, surface properties, and water solubility. In addition to solubility, crystal structure, surface charge and coatings, and conduction band energies are recommended as useful PC properties. They also recognise that one group should be identified by multiple types of parameters related to e.g. shape, biopersistence and toxicity; and that grouping could also be based on the potential of NMs to cause inflammation; dose is expressed as particle surface area for deriving dose-response curves from animal studies. Later in a follow up it was admitted that nanotubes should be considered as a separate group (Schröder et al. 2014). In a recent case study on grouping of NMs according to their ecotoxicological effects, reactivity, solubility, and morphology are identified as relevant factors in grouping metal and metal oxide NMs (Hund-Rinke et al. 2018).

The International Cooperation on Cosmetics Regulation (ICCR) supports the application of the

'bridging toxicity approach' in test waiving, which can be considered as implicit read-across. This consists in extrapolating (long-term) toxicity data between nanoforms or from a non-nanoform to a nanoform when the properties of the (non)-nanoforms and the results of the (short-term) toxicity studies are similar (Araki et al. 2013).

In occupational safety, the inhalation route is the exposure pathway of most concern. Some grouping schemes focus on PC properties relevant for the inhalation exposure route. For example, Gebel et al (2014) consider three categories according to the mode of action and exposure route: chemically mediated toxicity (e.g. soluble NMs), granular biodurable particles and fibrous NMs. Similarly, the German Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA 2013) distinguishes soluble particles, granular biopersistent particles with specific toxicological properties, granular biopersistent particles without specific toxicological properties, and biopersistent fibrous material.

The US National Institute on Occupational Safety and Health (NIOSH) has also followed a comparable grouping approach and in addition, has proposed a framework based on the mechanism causing the toxic effect. NMs are classified as higher solubility particles that can reach systemic tissues (toxic ions reach systemic tissue); poorly soluble, low toxicity[2] particles; poorly soluble, high toxicity particles (same as above but with reactive surface); fibrous particles for which the toxicity is related to biopersistence and genotoxicity (Kuempel et al. 2012).

The identification of these four classes of hazardous NMs is taken into account also by the British Standards Institution (2007). The ECETOC task force on NMs (Arts et al. 2015) released DF4nanoGrouping, an approach to group NMs for inhalation exposure in one of four main hazard classes following the above-mentioned categories (BAuA 2013; British Standards Institution 2007; Kuempel et al. 2012; Warheit Brown, and Donner 2015), thus distinguishing soluble NMs, biopersistent with high aspect ratio NMs, passive NMs, and active NMs.

This is done through a three-tier approach. Tier 0 precedes the DF4NanoGrouping by collecting intrinsic material properties to identify a NM. Tier 1 involves the assignment of a NM to the group of soluble NMs or to one of the other groups by means of its intrinsic properties. Tier 2 assigns the NM to one of the three groups (i.e. biopersistent high aspect ratio, passive, or active NMs) depending on its system-dependent properties. Toxicological information is used in Tier 3 to corroborate the assignment of the NM to a class and to support sub-grouping of active NMs depending on the outcome of short-term *in vivo* studies. The applicability of the framework is addressed by Arts et al. (2016). DF4NanoGrouping recommends read-across within the identified categories, consisting of NMs with similar PC and activity properties. For instance, group 1 may allow read-across between soluble NMs of the same chemical composition (even from bulk), group 2 for biopersistent and high aspect ratio NMs like CNTs, group 3 for non-fibrous passive NMs, and group 4 between reactive NMs, when possible, as this group may include different modes of action. The presented case studies cover 24 NMs of different classes of composition (carbonaceous, metal oxides and sulfates, amorphous silica, and organic pigments). Each identified NM was assigned to one of the four pre-defined groups following the three-tier approach. Assignment of NMs to groups 1–3 does not need animal testing, probably because the mode of action is clear from such grouping, whereas group 4 represents hazards that are addressed more specifically with *in vivo* experiments. Although DF4nanoGrouping framework defines qualifiers for grouping related to the use, release, and route of exposure, these considerations are not explicitly stated in the practical examples on carbonaceous NMs, metal oxide and metal sulphate NMs, amorphous silica, and organic pigments, as the presented case study is 'unrelated to exposure.'

Another type of approach is proposed by Hansen, Jensen, and Baun (2014) in NanoRiskCat, where NMs are categorised by taking into consideration shape (high aspect ratio NMs are considered top priority in terms of hazard) or evidence of toxicological effects (e.g. acute toxicity, genotoxicity, mutagenicity, and carcinogenicity). NANoREG screening risk assessment tool also identifies high aspect ratio (shape), and reactivity as relevant properties for human health hazard assessment (Dekkers et al. 2016).

Other authors apply high throughput screening platforms together with computational methods for data evaluation to rank NMs and to guide *in vivo*

testing. For example, Nel et al. (2013) have identified a set of *in vitro* assays reflecting toxicity pathways of NMs. The tests provide information about ROS, dissolution and release of toxic metal ions, cationic injury to surface membrane and organelles, profibrogenic responses to CNTs, inflammasome activation by long aspect ratio materials, photoactivation and influence of bandgap, Zebrafish embryo hatching interference, or cell membrane lysis by surface reactivity. The resulting data is claimed to support clustering based on similar biological responses or linkage to PC properties (e.g. shape, size, crystal structure, band gap, dissolution, surface chemistry, surface charge, and surface functionalisation), but this is not translated into practical guidance.

The RIVM approach (Sellers et al. 2015) consists of different steps that aim at substantiating a grouping hypothesis taking into account the behavior and toxicity of the NM of interest. A tiered testing strategy is presented where data are collected at different levels of complexity (tiers 0–2; some pieces of information are not required by REACH but are considered necessary for the assessment) and read-across is considered for each endpoint according to similarities identified depending on collected information (mainly on PC properties and behaviour in environmental or biological media). The proposed strategy consists of a four-step framework and on a three-tier data collection to evaluate NMs and decide on the applicability of read-across. The four steps comprise:

1. Collection of existing information (including NM characterisation and behaviour of the NM in different media).
2. Hypothesis formulation.
3. Testing (3 tiers: PC properties, reactivity and *in vitro* toxicity, and *in vivo* toxicity).
4. Assessment (do data support the hypothesis, or is there need for new data?).

Step 1 is used to collect data to form a hypothesis (step 2) that may lead to experimental testing, which is used to issue a final assessment. The framework is illustrated by its application to two generic NMs (nano-Ag and nano-TiO_2); more emphasis on NM identification would probably be necessary in a real case application. This approach does not aim primarily at assigning a NM to a predefined category, as hazard groups are eventually defined in a flexible manner after collection of information on PC properties and toxicological endpoints. In this approach, the LC of products containing NMs is considered as a step for identifying exposure routes when addressing specific case studies. Further testing may be required in case the data do not support the grouping hypothesis.

Information on the 'assessment goal' of the grouping approaches has been gathered in Table 1. To support the discussion section on the different available grouping frameworks and case studies, we extract the basic principle applied for grouping ('Basis for grouping') and identify 'predefined groups.' In case a 'testing strategy' is supported, then this is pinpointed in a dedicated column. We also report if the approach identifies the availability of standard methods like OECD test guidelines or other standard operating procedures (SOPs), based on the considerations made by the authors ('Practicality') and if applications to case studies are presented ('Applicability'). Finally, we comment on the applicability of the proposed method for REACH purposes in the last column.

Hazard classes in control banding tools

Control banding (CB) is a pragmatic approach that can be used for the control of the workplace exposure to agents with unknown or uncertain toxicological properties and for which there is a lack of quantitative exposure estimations. CB tools identify a range of control measures (such as general ventilation and containment) according to the estimated range or 'band' of hazard and of exposure based on combined hazard and exposure ranking.

For the purposes of this review, we were interested in investigating which PC properties or which toxicological endpoints are applied to rank the hazard of NMs in various nanospecific CB tools, as this may in some cases also support the grouping NMs for read-across of hazardous (toxicological) properties.

Liguori et al. (2016) published an extensive review of all the available CB tools applicable to NMs, and comparing them in terms of the required inputs of PC properties, toxicology, and exposure. We have extracted from this review to Table 2 information on the PC properties taken into consideration in the hazard ranking of NMs in the different

CB tools. The PC properties reported in the table are the key features of the available CB tools specific to NMs. The aim of each CB tool is reported under 'assessment goal,' whereas 'PC properties considered for hazard classification' contains details on the PC properties considered in the hazard banding; in case toxicological information is taken into account or required by the tool this is also reported in this column. Under the column 'hazard classes' details on the number and type of hazard bands are reported and finally under 'availability of case study' reference to any applications is reported. From the table, it is evident that solubility, together with shape, are considered relevant PC properties for identifying the hazard group in all the tools except the Swiss precautionary matrix, where it was excluded because of lack of data (Höck et al. 2013). Solubility (either in water or in solutions at different pH values thus representing a biological environment) is taken into account in most tools as a screening property: the biological effects of highly soluble materials are considered similar to coarser particles and traditional risk assessment tools are considered suitable in these cases. For instance, in the Stoffenmanager tool, hazard banding is based on water solubility and biopersistence (highly soluble particles are considered lower priority substances) (Van Duuren-Stuurman et al. 2012). On the other hand, the ANSES CB Tool requires solubility rate in water or in biological medium as an input and in case of low solubility rate, the assigned hazard band is higher (ANSES 2010; Liguori et al. 2016). Surface coating is a required input for hazard banding only in Nanosafer, and can be taken into consideration according to its stability in the Swiss precautionary matrix (Höck et al. 2013). In some tools, biopersistence is considered as well (Liguori et al. 2016).

Both NanoSafer and the ANSES CB tool consider the possibility to take into account data from the corresponding bulk material or analogous material. In the ANSES CB Tool an analogous material is defined as 'a substance or material with a similar composition and/or crystalline phase from the same chemical category and with similar documented physicochemical properties (metal oxides, graphite, ceramics, etc.) as the substance of interest' (Riediker et al. 2012).

NANOSOLUTIONS developed a classifier of NM toxicity (ENM safety classifier) based on multiple data sources (intrinsic properties, omics data and *in vitro* toxicity data generated from the project or coming from other databases). Although the classifier is not developed as a CB tool, it is reported in Table 2 because it identifies three hazard classes that are defined according to PC properties and toxicological tests (Fortino and Grevo 2017; NANOSOLUTIONS 2017).

Oosterwijk et al. (2016) propose a conceptual framework for inhalation exposure, that can be updated and validated once more information on the property–toxicity relationships and on the NMs mechanism of action become available. The hazard module computes different scores according to the accumulation fractions of NMs in different respiratory regions (nasal, tracheobronchial, and pulmonary region) and according to local or systemic toxicity that is considered in the alveolar region. PC properties such as net charge, size, hydrophobicity/hydrophilicity, solubility and ion toxicity, and conduction band energy are computed to classify the NMs into four hazard classes. Drew et al. (2017) proposed a quantitative framework to group NMs and bulk materials through a set of properties including density, surface area, and diameter that were most predictive of the potency to elicit neutrophilic pulmonary inflammation (acute exposure).C.,

In a data-poor context, Bayesian networks have been proposed by Marvin et al. (2017) for human hazard ranking of NMs. This approach includes the selection of PC parameters relevant for hazard assessment of NMs by expert elicitation, and in the construction of a Bayesian network to classify NMs according to the information on exposure and hazard. A validation exercise shows that the ranking of hazard potential of NMs was satisfactory. As the authors state, the proposed model presents probabilistic relationships among a set of variables and draws conclusions based on available information and expert knowledge. The limit of this approach is that there is no mechanistic evidence in hazard identification, as hazard is linked to the eliciting property depending on probability and information on mode of action is not considered explicitly in the model. Since this model is not applied as a CB approach, it is not included in Table 2.

Application of computational tools in grouping for read-across of NMs

Research activities have been carried out not only to develop frameworks to group NMs, but also to assess them in case studies for grouping of NMs based on similarities. Our search revealed that only a few studies successfully attempted to identify similarity to justify grouping of NMs, and these are listed in Table 3. Since there are only a few examples of grouping of NMs in the literature, in our analysis also other relevant approaches are included and summarised (Table 3). In particular, approaches that result in the ranking of NMs are potentially useful since a rank ordering of chemicals (or NMs) may allow a group to be defined, and interpolation (i.e. read-across) of properties can be carried out between NMs of known toxicity.

In a recent case study by Lamon et al. (2018; OECD, 2018) the ECHA workflow for grouping and read-across of NMs was applied to a data-rich NM (TiO_2). A grouping hypothesis was made based on the mode of action of TiO_2 nanoforms and chemoinformatic techniques such as hierarchical clustering, principal components analysis, and random forest for variable selection were used to support grouping and identify key PC properties to predict the *in vitro* comet assay results of the target substances. The case study used PC properties collected from available studies (OECD, 2015) for six source analogues, and two target nanoforms were considered for the read-across (Guichard et al. 2012). The grouping hypothesis was based on the presence of coating and impurities, which was found to correlate with negative *in vitro* comet assay results indicating that they may act by preventing the contact between the nano-TiO_2 and the cellular components or DNA.

Another case study showing an example of read-across application for NMs for filling data gaps is presented by Gajewicz et al. (2015). They consider two different case studies: *in vitro* cytotoxic endpoints for *Escherichia Coli* (17 metal oxides) and a human keratinocyte cell line (18 metal oxides), and they calculated descriptors for activity (enthalpy of formation of a gaseous cation having the same oxidation state as that in the metal oxide structure and Mulliken's electronegativity). Euclidean distance was the similarity metric applied for the identification of groups of NMs with similar toxicity. Data were split into training and validation sets to perform read-across. The 'prediction' was successful from both sets except for a few oxides for which toxicity is under predicted (SnO_2, Mn_2O_3, and V_2O_3) in the HaCaT cell line for TiO_2.

Sizochenko et al. (2018) apply cluster analysis and self-organising maps with the same objective to identify groups of NMs with similar toxicity for read-across. This case study is also based on *in vitro* IC50 and EC50 data on different cell lines (from *Escherichia coli*, *Photobacterium phosphoreum*, and *Vibrio fischeri*, human keratinocyte cell line HaCaT, epithelial cell line A549, human epithelial colorectal cell line Caco2, murine fibroblast cell line Balb/c 3T3, a microalga *Pseudokirchneriella subcapitata*, and protozoan *Tetrahymena thermophile*). Twelve groups of NMs are identified, corresponding to three toxicity classes (low, medium, and high), plus one class corresponding to unknown toxicity. The most important parameter in predicting toxicity is the enthalpy of cation formation and this is applied for data-gap filling that leaves some of the predicted values out of range.

Zhang et al. (2012) identify different hazard groups of NMs according to dissolution and conduction band profiles. The conduction energy band prediction was successful in confirming the toxicity of the metal oxides whose band gap was overlapping with the cellular redox potential. Other metal oxides exhibiting toxic effects were outside the band gap range identified by the model (false negative predictions), and to solve this, a regression tree analysis taking into account both the effect of band gap and dissolution was successfully used to identify three groups of NMs: non-toxic NMs, NMs toxic because of their solubility, and NMs toxic because of their conduction energy band. Tested doses are not normalised to NMs surface area, but are expressed per volume.

A different approach for ecotoxicity was proposed by Hund-Rinke et al. (2018) where metal and metal oxide NMs are grouped according to three properties that were considered relevant: ion release, morphology, and reactivity. PC characterisation of NMs is provided in the paper (surface chemistry, surface area, crystalline structure, morphology, primary particle size, hydrodynamic diameter, zeta potential, isoelectric point, and solubility rate). They

apply statistical methods to a dataset of EC50 values for algae, Daphnids, and fish embryo for 14 NMs (five chemical compositions, different size, shape, crystallinity, solubility, and reactivity). They identify six groups: reactive ion releasing NMs, wires; non-reactive ion releasing NMs, wires; reactive, ion releasing NMs with different morphology; non-reactive, ion releasing NMs, other morphology, reactive, non-ion releasing NMs, other morphology; non-reactive, non-ion releasing NMs, other morphology. The authors then apply the scheme to a set of NMs that were not included in the 'training set' for grouping (SiO_2, TiO_2, and Fe_2O_3). The application confirms that NMs with the same chemical composition fall in the same group.

Discussion

This article reports and discusses the available grouping approaches that have been developed for NMs. Tables 1–3 present different types of grouping approaches available in the literature with different assessment goals. In some cases, grouping frameworks are developed with the specific aim to waive animal testing by applying a tiered approach aimed at assigning NMs to a hazard class to read-across data in the same hazard category (e.g. DF4NanoGrouping) (Table 1). Tools supporting 'grouping' of NMs according to identified PC properties are also reported to provide a list of PC properties that are considered relevant in classifying NMs as toxic or non-toxic (Table 2). Other studies selected for this review focus on the feasibility of applying grouping approaches strictly following the REACH definition on reading across hazard endpoints in Annex XI of the regulation (Table 3). In the following paragraphs, we compare the different approaches and identify commonalities and differences in the outcomes.

The approaches reported in Table 1 aim at identifying *a priori* PC properties to group NMs in hazard classes, thereby supporting read-across within that group. Most frameworks reported in Table 1 identify PC properties such as solubility, shape, and surface properties (surface chemistry or reactivity) biopersistence and reactivity as relevant for grouping (Arts et al. 2015; Kuempel et al. 2012; Oomen et al. 2015; RCC 2013a, 2013b). Only one approach presented in Table 1 takes into consideration information on exposure and emissions related to the stages of the product's life cycle (Arts et al. 2015), thus following the idea of including release and exposure information during the LC of NM-containing products in grouping approaches considered in risk assessment (Stone et al. 2013). The importance of morphology in the identification of the groups is explained by the fact that most toxicity studies available are inhalation case studies, and in fact most of the proposed frameworks are generated on inhalation data (Arts et al. 2015) or are applied in the field of occupational risk assessment (Kuempel et al. 2012).

On the other hand, the framework outlined by RCC (RCC 2013a, 2013b) identifies groups depending on the NM's core chemical composition. Interestingly, only one of the approaches reported in Table 1 (DF4nanoGrouping, Arts et al. 2015) reports on the availability of accepted standard methods for the identified PC properties; in general, the other approaches report on a lack of SOPs. For DF4nanoGrouping case studies were performed to demonstrate the applicability of grouping NMs (Arts et al. 2016) in the proposed hazard categories. This framework, together with the grouping approach presented by ECHA (2017) was selected as a reference for development in the H2020 project *Grouping, Read-Across, CharacterIsation and classificatiOn framework for regUlatory risk assessment of manufactured NMs and Safer design of nano-enabled products* (GRACIOUS)[3] which started in January 2018.

Table 2 shows the identification of NM PC properties in occupational banding tools that were included in this review because they identify hazard levels assigned to each NM for computing it against an exposure value to predict the risk in occupational settings. The relevance of these approaches lies in the selection of PC properties for hazard classification, and the identification of hazard classes that in some cases are characterised by specific NM properties. Regarding the PC properties, morphology and water solubility are considered in most of the approaches (ANSES 2010; Cornelissen et al. 2011; Jensen et al. 2014; Oosterwijk et al. 2016; Van Duuren-Stuurman et al. 2012; Zalk et al. 2009). Reactivity is considered as surface reactivity (ANSES 2010; Zalk, Paik, and Swuste 2009) or as ROS formation potential (Höck et al. 2013), whereas surface

coating (or surface chemistry) is considered only in one approach (Jensen et al. 2014) in hazard evaluation and in one approach in Table 1 (RCC 2013a, 2013b) and also in Table 3 (Lamon et al. 2018). Coating stability is not required as a nanospecific property and hence this information is not available from the studies reported in this review. Höck et al. (2013) consider the coating stability as a distinctive property to build a matrix only for the coated or core NM, or both forms according to the solubility rate of the coating. However, the authors do not take into consideration coating stability for ranking of NMs. Biopersistence is considered in two approaches (ANSES 2010; Van Duuren-Stuurman et al. 2012). Only one model proposes hazard classes that are defined according to solubility, biopersistence and shape (Cornelissen et al. 2011).

Finally, Table 3 shows available approaches where grouping for read-across of toxicological endpoints is proposed through the application of computational methods to identify similarity. There are only a few studies of this type available in the literature, and most of them interestingly select non-specific PC properties as relevant predictors to read-across to target NMs. Only five studies reported in Table 3 substantiate read-across by identifying similarities based on PC properties (Gajewicz et al. 2015; Lamon et al. 2018; Sizochenko et al. 2018; Zhang et al. 2012). In the studies from Gajewicz et al. (2015), Zhang et al. (2012) and Sizochenko et al. (2018) the dataset does not include nanospecific PC properties[4], and chemical descriptors are used for the read-across prediction (enthalpy of formation of a gaseous cation having the same oxidation state as that in the metal oxide structure and Mulliken's electronegativity in Sizochenko et al. (2018) and Gajewicz et al. (2015). Zhang et al. (2012) identify the conduction energy band as a predictor in cytotoxicity and the solubility of the metal oxides as a predictor in acute inflammatory response in the lungs. The case study presented by Lamon et al. (2018) considers specific NM PC properties, and the presence of surface coating or impurities is selected as a property affecting the result of the *in vitro* comet assay of the tested NMs. Regarding the selected endpoints for read-across, none of the case studies available in Table 3 consider REACH-relevant endpoints[5]. A case study recently published by Hund-Rinke et al. (2018) where metal and metal oxide NMs are grouped for aquatic toxicity according to ion release, morphology, and reactivity, takes finally into consideration a REACH-relevant endpoint. This aspect conflicts with the grouping frameworks study reported in Tables 1 and 2, where hazard data are related to animal studies and to apical endpoints. This is explained by the lack of accessible databases, as reported thoroughly by Worth et al. (2017).

Addressing similarity

Considering REACH Annex XI, similarity needs to be justified when grouping to read-across hazard endpoints; computational methods may be useful in supporting justification of a grouping hypothesis. This principle is applied in most approaches reported in Table 3. PC properties that have been found to predict toxicity successfully can be divided into chemical (energy-related properties of the chemicals considered in the case study) or nanospecific PC properties (e.g. surface chemistry, reactivity, and morphology). The commonality between all the case studies reported in Table 3 is the relevance of reactivity-based parameters (enthalpy of formation of a cation, release of ions) as these parameters are key in predicting the toxic action of the metal or metal oxide NMs, so when reading across toxicity endpoints it is relevant to test reactivity properties of the material e.g. through *in vitro* tests (Hund-Rinke et al. 2018). On the other hand, as most authors admit, the datasets of PC properties considered are quite limited and it would be necessary to investigate larger datasets to identify a relevant, exhaustive set of relevant PC properties or of common mode of action or biological process (OECD, 2014) justifying similarity in grouping for read-across of NMs. An original contribution on how to deal with similarity is provided in the NM registry by the US National Institute of Health (NIH) with defined similarity rules to support matching of NM entries in the registry (NIH 2014). Such rules determine similarity in the range 10–85% depending on surface chemical composition, surface charge, shape, and size. If the NMs were characterised in the same environment (defined taking into consideration both the kinetic and thermodynamic aspects) for size, then the NMs are in a 22.5–30% match; if the size values are within 10%, those two

NMs have an additional 15% match. If both NMs have the same material type for their most outward chemistry, they have an additional 25% similarity, and if the isoelectric point value is within 10% and the NMs were characterised in the same way, another 15% similarity can be added (NIH 2014). These similarities are identified upon the NIH database and they can be updated according to new data.

As reported in this manuscript, there are a few studies addressing NM similarities, but these are based on limited datasets. Further work should investigate toxicity datasets and PC nanospecific properties to identify groups of NMs; the approach presented by Hund-Rinke et al. (2018) that propose specific assays to investigate reactivity aspects is a possibility that could be extended to many other applications for different endpoints.

Grouping for read-across is envisaged in European legislationto reduce animal testing; accordingly, a grouping hypothesis would be expected to be substantiated for apical endpoints taken into consideration in REACH (e.g. acute toxicity and repeated dose toxicity). Only one of the case studies reported in Table 3 builds on *in vivo* tests, which are applied to justify the alternative testing paradigm proposed by the authors that include *in vitro* and *in silico* methods (Zhang et al. 2012). In this case, toxicokinetics are important to justify grouping of NMs, for which SOPs are needed. Efforts are ongoing to develop databases where toxicity data and PC properties are collected in one place, in order to improve data availability and enable investigations supporting grouping for read-across and development of *in silico* methods (Chen et al. 2017). eNanoMapper (Jeliazkova et al. 2015) is supporting read-across applications and *in silico* modeling, and the running project GRACIOUS is defining a grouping framework that takes more explicitly into account similarity.

Research gaps

As described above, there are different types of grouping approaches available in the literature. The tools proposed in Table 1 were developed to support grouping for read-across in REACH regulatory submissions. In these approaches, a grouping hypothesis is defined in the first place, and the framework helps applicants for further application of the groups to other NMs that fall within the same group. This type of approach is user friendly since on the basis of a few PC properties (e.g. morphology, solubility, and reactivity) it should be possible to group NMs and to apply read-across. However, the lack of considerations on similarity and on its justification may not be sufficient to be accepted for their use in a regulatory context, e.g. REACH. Several publications (Gajewicz et al. 2018; Scott-Fordsmand, Amorim, and S⊘rensen 2018) aim at supporting the application of DF4Nano to explore relationships between NM PC properties and toxicity endpoints. On the other hand, the approaches reported in Table 3 are taking into consideration similarity on a case-by case basis: depending on the case studies, the dataset spans different chemical compositions or the same core NM (i.e. same chemical composition) with differences in surface chemistry, size, and morphology. The drawback of these types of approaches is that they are not as user-friendly as those reported in Table 1. Efforts are being made by the scientific community to develop a grouping framework that takes more explicitly into account similarity while retaining some flexibility on the identification of the groups: the H2020 GRACIOUS project will build on previous results from the projects NanoREG, GUIDEnano, and Calibrate to go in this direction.

Regarding the PC properties identified as relevant in grouping approaches reported in this review and the need to justify a grouping hypothesis based on similarity, it is interesting to point out that the Working Party on Manufactured Nanomaterials (WPMN) (OECD, 2009) concluded that only 4 out of 22 test guidelines for measuring PC properties are applicable to NMs. In the last 10 years progress has been made in terms of identification of relevant PC properties and SOP development as thoroughly described by the ProSafe project (Rasmussen et al. 2018; Steinhäuser and Sayre 2017) which presents an up-to-date selection of PC properties and the preferred methods available for their measurement. The availability of SOPs for measuring PC properties is key to have a dataset of comparable measurements for model development, including grouping for read-across. SOPs are missing for the NM properties highlighted in this review as relevant for NM grouping. In particular,

there is no standard protocol to measure the (rate of) solubility and biopersistence, which are reported as relevant PC properties for grouping in different types of grouping approaches (Tables 1–3).

However H2020 proposals have developed SOPs and infrastructures for their dissemination, like NanoReg[6] that elaborated SOPs on hydrodynamic size distribution (Mast and De Temmerman 2016), solubility/dissolution and biodurability and hydrochemical reactivity; some of these are an item in CEN for development of technical specification in the test methods (Stone et al. 2017), and also developed the NANoREG Toolbox, an infrastructure collecting test methods, datasets and models that support the safety assessment of NMs (Jantunen, Gottardo, and Crutzen 2017). NANoREG 2 is giving continuity by building safe-orientated grouping approaches linked with Intelligent Testing Strategies (ITS) and disseminating Safe-by-Design tools and SOPs.

Conclusions

Currently available and diverse grouping approaches for NMs to read-across hazard endpoints are reported in this review. The PC properties that are relevant to support grouping for specific types of hazard are analysed and discussed. We make a clear distinction between the more standardised approaches that tend toward the definition of a workflow where a NM is classified through a set of fixed PC properties in a hazard group (Tables 1 and 2), and the more exploratory approaches that compute a dataset of NMs to investigate any similarity to justify grouping for read-across of the identified endpoint (Table 3).

On the approaches reported in Table 3, only one takes into account nanospecific properties (Lamon et al. 2018) and only one study takes into account apical endpoints (Zhang et al. 2012).

Definition of SOPs and of batteries of *in vitro* and PC properties testing to address the mode of toxic action of NMs depending on the endpoint of interest (e.g. Hund-Rinke et al. 2018, Bove et al. 2017), and data sharing may improve the availability of case studies including nanospecific properties and applied for apical endpoints of interest. On the other hand, data quality and comparability must be guaranteed by application of SOPs and by the definition of quality criteria: the Dana initiative started to work in this direction (Marchese Robinson et al. 2016), and the recently started H2020 project GRACIOUS is following this direction.

Notes

1. Grouping of nanoforms of the same substance is addressed in draft REACH Annexes released for consultation in October 2017 (https://ec.europa.eu/info/law/better-regulation/initiatives/ares-2017-4925011_en)
2. toxicity is related to total deposited or retained particle dose in the target respiratory tract region based on particle size.
3. https://cordis.europa.eu/project/rcn/212339_en.html
4. In this manuscript by nanospecific properties we refer to PC properties identified specifically for NMs in OECD harmonised templates adopted in IUCLID for REACH submissions: agglomeration/aggregation, crystalline phase, crystalline and grain size, aspect ratio/shape, specific surface area, zeta potential, surface chemistry, etc. (http://www.oecd.org/ehs/templates/harmonised-templates-physico-chemical-properties.htm)
5. acute toxicity, irritation, corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity, aquatic toxicity, and effects on terrestrial organisms are REACH (eco)toxicity endpoints.
6. For an overview of released SOPs please consult the project dissemination webpage: http://www.rivm.nl/en/About_RIVM/Mission_and_strategy/International_Affairs/International_Projects/Completed/NANoREG/Work_Package

Acknowledgments

This work was part of the Nanocomput project, carried out by the European Commission Joint Research Centre (JRC) for the Directorate-General (DG) for Internal Market, Industry, Entrepreneurship and SMEs (DG GROW) under the terms of an Administrative Arrangement between JRC and DG GROW.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The project was funded by DG GROW of the European Commission under the terms of a Service Level Agreement between DG GROW and the JRC. The work was supported by a steering group with representatives from DG GROW, DG Environment and the European Chemicals Agency (ECHA).

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