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# Regulation of engineered nanomaterials: current challenges, insights and future directions

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**Abstract** Substantial production and wide applications of engineered nanomaterials (ENMs) have raised concerns over their potential influences on the environment and humans. However, regulations of products containing ENMs are scarce, even in countries with the greatest volume of ENMs produced, such as the United States and China. After a comprehensive review of life cycles of ENMs, five major challenges to regulators posed by ENMs are proposed in this review: (a) ENMs exhibit variable physicochemical characteristics, which makes them difficult for regulators to establish regulatory definition; (b) Due to diverse sources and transport pathways for ENMs, it is difficult to monitor or predict their fates in the environment; (c) There is a lack of reliable techniques for quantifying exposures to ENMs; (d) Because of diverse intrinsic properties of ENMs and dynamic environmental conditions, it is difficult to predict bioavailability of ENMs on wildlife and the environment; and (e) There are knowledge gaps in toxicity and toxic mechanisms of ENMs from which to predict their hazards. These challenges are all related to issues in conventional assessments of risks that regulators rely on. To address the fast-growing nanotechnology market with limited resources, four ENMs (nanoparticles of Ag, $TiO_2$, ZnO and $Fe_2O_3$) have been prioritized for research. Compulsory reporting schemes (registration and labelling) for commercial products containing ENMs should be adopted. Moreover, to accommodate their potential risks in time, an integrative use of quantitative structure-activity relationship and adverse outcome pathway (QSAR-AOP), together with qualitative alternatives to conventional risk assessment are proposed as tools for decision making of regulators.

**Keywords** Risk assessment framework · Environmental fate and behaviour · Pre-market evaluation · Quantitative structure–activity relationship · Adverse outcome pathway · Alternatives risk assessment framework

## Introduction

Nanomaterials are materials with one or more dimensions in the nano-scale (1–100 nm; one billionth of a meter; $1 \times 10^{-9}$ m), characterized by their enhanced reactivity and emerging physicochemical properties compared with their corresponding bulk forms (Nel et al. 2006). Nanomaterials can come from various sources. Natural sources include bacterial metabolism, hydrothermal vents and volcano dust (Fig. 1, Farré et al. (2011)). Engineered nanomaterials (ENMs) from anthropogenic sources are designed with unique properties. Therefore, compared to the natural forms, ENMs have distinctive interactions with biotic and abiotic

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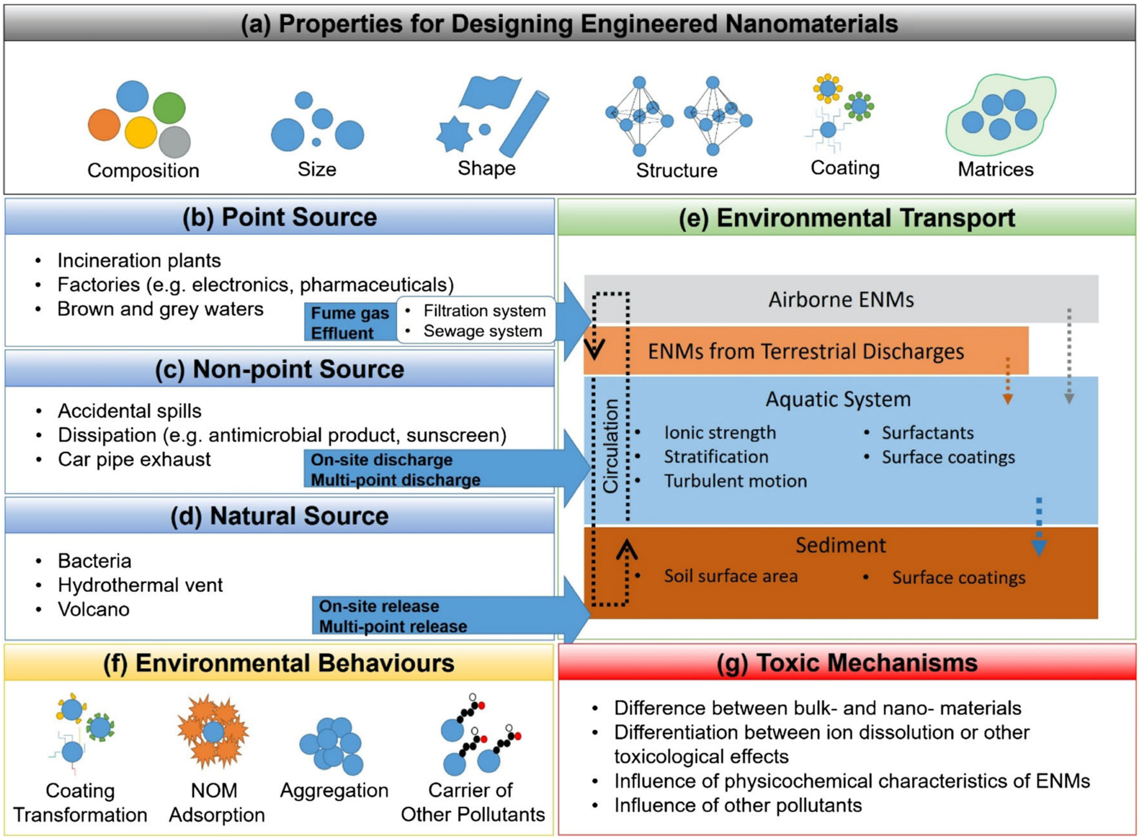

**Fig. 1** A schematic diagram summarizing possible environmental behaviours and fates of engineered nanomaterials

constituents of the environment (Handy et al. 2008). Enhanced characteristics of ENMs include optical, thermal and electrical properties, material strength, conductivity, solubility and reactivity (CFS 2010; Klain et al. 2008; Kumar 2006). These unique properties of ENMs result in widespread applications in commercial products, and make benefits for various industrial sectors, such as food, cosmetics, pesticides and pharmaceuticals. It is predicted that global market value of ENMs will reach US$ 11.8 billion by 2025 (Mordor Intelligence 2016).

Inevitably, burgeoning development and broad applications of ENMs will result in releases to the environment with concomitant exposures to wildlife and possibly humans (Beaudrie and Kandlikar 2011). Concerns have been raised over their potential influences in the environment but uncertainties and ambiguities remain, that have resulted in controversies over the sustainable use and safety of ENMs. Besides the dilemma between proclaimed benefits and risks posed by use of nanotechnology (Hansen et al. 2008a), technical problems occur with respect to assessing their toxic effects and mechanisms, which might or might not be different from their bulk counterparts (Hansen and Baun 2012). Nevertheless, lessons have been learnt from examples such as asbestos, dichloro-diphenyl-trichloroethane (DDT), chlorofluorocarbons (CFCs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and tributyltin (TBT) that how costly it will be if potential unintended and unforeseen consequences of new technologies occur (EEA 2001; Hansen et al. 2008a). Therefore, it is important to review deficiencies of current regulations since it is possible that current guidelines and regulations developed for bulk materials might not be suitable for their corresponding ENMs and hence might overlook their influences, which could result in widespread adverse outcomes that are difficult to reverse. It is also critical to have more research effort for scrutinizing potential risks of ENMs to provide information for regulators, so as to protect humans and the environment.

To narrow the scope of this review, it focuses on ENMs that come from anthropogenic sources and have relatively larger contribution to environmental contamination than natural nanomaterials (Farré et al. 2011). Influences of ENMs in the aquatic environment and associated sediment, which are final sinks for most of the terrestrial pollutants, are reviewed. The review is divided into four sections: (1) Currently available regulations on ENMs in various regions and countries are summarized; (2) Five key challenges in risk assessment of ENMs are proposed as hindrances to development of regulations; (3) Based on their respective annual production, market share in terms of commercial products and toxicity, ENMs that require prompt investigation and regulation are prioritized; and (4) Prompt qualitative administrative means are proposed to gather more information for traditional risk assessment framework, and the concept from the alternatives assessment framework that tolerates, but identifies, uncertainties is proposed to implement future regulations on ENMs. Through these, the review can provide an understanding in current deficiencies and key limitations to the development of regulations for ENMs. The final goal is to concentrate limited resources on risk assessments of ENMs and provide information for establishment of adequate regulations in time.

## Regulations on commercial products containing ENMs

### Current regulations in different countries

Despite rapid growth and application of nanotechnology, relevant regulations on ENMs are limited. Herein, regulations in six countries of four commercial product sectors are reviewed **(**Table 1**, Online Resource section 1)**. The four sectors are food, pharmaceuticals, cosmetics and pesticides, which apply ENMs in large quantity and a total of 27 relevant regulations have been reviewed. Regulations are summarized into several categories including regulatory definition of ENMs, reporting mechanism (labelling) on products containing ENMs, guidance for evaluation of risk, and requirement of pre-market registration or evaluation of products. These are all important components in a regulatory guideline for management of chemical substances (Beaudrie et al. 2013; Hansen and Baun 2012; Linkov et al. 2009).

Definitions of ENMs are mostly provided in regulatory guidelines except in China, Japan, and sectors of pharmaceuticals and food in Australia, as well as sectors of pharmaceuticals and pesticides in the United States (Table 1). Similarly, the aforementioned sectors, except for the food sector in Australia and cosmetics sector in Japan, do not have compulsory labelling systems for these products. However, for compulsory labelling schemes in other sectors, neither of them requires specification of ENMs in commercial products, except the sector of cosmetics in the European Union. Furthermore, only a few sectors in the European Union and the United States provide standardized guidance for characterization, toxicity test, risk assessment and proper application of commercial products containing ENMs. Pre-market registration or evaluation of products is required in many countries, but about half of them adopted existing approaches without addressing ENMs.

### Potential problems of current regulations

Currently no comprehensive framework for regulation of ENMs is available. Countries such as the United States and China, which are major producers and consumers of ENMs (Brazell 2012), are paying relatively little attention to regulations for use of ENMs (Table 1). Since most of the compulsory labelling schemes do not recognize ENMs, customers and other interested parties will have little acknowledgement on commercial products containing ENMs and hence soft laws through public monitoring might not be practical (Linkov et al. 2009; Malloy 2011). Although many of the reviewed sectors require compulsory pre-market evaluation, those without specification of ENMs might not be effective to accurately assess the risk of ENMs. For instance, if guidance for assessment does not distinguish between nano- and bulk materials, some hazardous ENMs might pass pre-market evaluations when their bulk counterparts are classified as non-toxic (Bondarenko et al. 2013). This is possible since it has been suggested that chemicals can become more toxic when they are in nano-scale (Nel et al. 2006). Even during pre-market evaluations that recognize ENMs, the definition of ENMs is not provided (e.g. Japan), such that it might hinder manufacturers and regulators from evaluation. Case by case evaluations (e.g. Japan) might not be realistic when nanotechnology is being promoted in a growing and extensive trend (Beaudrie et al. 2013). Furthermore, results from pre-market evaluations of commercial products by various jurisdictions might not be reliable or comparable because there is little standardized guidance for specialized assessments of ENMs, while some current guidance for bulk materials might not be suitable for evaluation of ENMs (Hansen et al. 2009; OECD 2009; SCENIHR 2007).

Another possible problem for regulations without specification of ENMs is their potential exemptions from regulation and evaluation because they cannot meet the threshold value tailored for bulk chemicals. For instance, under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), which regulates all chemical substances in the Europe Union, registration and assessments of chemicals are not required if they are produced or imported for less than 1 ton/year (ECHA 2016). ENMs might not be evaluated under this threshold because their volume produced and imported is generally smaller (Hansen and Baun 2012). Similarly, if concentrations of ENMs present in commercial products are less than 0.1% *w*/w, no safety information is required while such small concentrations are common for products containing ENMs (ECHA 2016; Linkov et al. 2009). Similarly, in the United States, the Toxic Substances Control Act gives Low Volume Exemption for substances

**Table 1** Regulations in different countries and regions have been grouped into several collective terms. "Definition" refers to any general or working definition used by the authority during the regulation. "Guidance on Characterization, Toxicity Test, Risk Assessment and Application" covers any developed guidelines that the authorities propose during the evaluation of the products. "Pre-market Evaluation" refers to pre-registration of commercial products when the active ingredients should be stated and evaluated before they are on sale to public. "Product Labelling" refers to notification of customers that the ingredients in the product through its package labels. Ticks in red colour and with '*' refer to application of existing approaches, i.e. manufacturers can follow current regulatory guidance, which are mostly for bulk materials, for their products containing corresponding nanomaterials. Sources of information include: (1) Industrial Chemicals (Notification and Assessment) Act 1989; (2) Therapeutic Goods Act 1989; (3) Australia New Zealand Food Standards Code; (4) Australian Pesticide and Veterinary Medicines Agency; (5) Nanotechnologies for pesticides and veterinary medicines: Regulatory considerations; (6) Food and Drugs Act; (7) Cosmetics Regulations C.R.C., c. 869; (8) Policy Statement on Health Canada's Working Definition for Nanomaterials; (9) Food and Drug Regulations C.R.C., c.870; (10) Pest Control Products Act; (11) Pest Control Products Regulations (SOR/2006–124); (12) Directive 76/768/EEC; (13) Regulation (EC) No 1223/2009; (14) Reflection paper on nanotechnology-based medicinal products for Human Use; (15) Directive 2001/83/EC; (16) Regulation (EC) No 178/2002; (17) Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain; (18) Regulation (EC) 1107/2009; (19) Directive 98/8/EC; (20) The Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices; (21) Ministry of Health, Labour and Welfare (22) Federal Food, Drug, and Cosmetic Act; (23) Guidance for Industry – Safety of Nanomaterials in Cosmetic Products; (24) Public Health Service Act; (25) Nanotechnology Task Force; (26) Guidance for Industry on the Use of Nanomaterials in Food for Animals; and (27) Federal Insecticide, Fungicide, and Rodenticide Act

| | Category | Regulations | Definition | Product Labelling | Guidance on Characterization, Toxicity Test, Risk Assessment and Application | Pre-market Reporting and/or Evaluation |
|---|---|---|---|---|---|---|
| **Australia** | **Cosmetics** | 1 | ✓ | ✓*<br>(Compulsory) | | |
| | **Drugs** | 2 | | | | |
| | **Food** | 3 | | ✓*<br>(Compulsory) | | ✓*<br>(Compulsory) |
| | **Pesticides** | 4, 5 | ✓ | | | ✓<br>(Compulsory) |
| **Canada** | **Cosmetics** | 6, 7, 8 | ✓ | ✓*<br>(Compulsory) | | |
| | **Drugs** | 6, 8, 9 | ✓ | ✓*<br>(Compulsory) | | |
| | **Food** | 6, 8, 9 | ✓ | ✓*<br>(Compulsory) | | |
| | **Pesticides** | 8, 10, 11 | ✓ | ✓*<br>(Compulsory) | | ✓*<br>(Compulsory) |
| **China** | | | | | | |
| **European Union (including UK)** | **Cosmetics** | 12, 13 | ✓ | ✓<br>(Compulsory) | ✓ | ✓<br>(Compulsory) |
| | **Drugs** | 14, 15 | ✓ | ✓*<br>(Compulsory) | | ✓<br>(Compulsory) |
| | **Food** | 16,17 | ✓ | ✓*<br>(Compulsory) | ✓ | |
| | **Pesticides** | 17, 18, 19 | ✓ | | ✓ | ✓*<br>(Compulsory) |
| **Japan** | **Cosmetics** | 20 | | ✓*<br>(Compulsory) | | ✓<br>(Compulsory) |
| | **Drug** | 20 | | | | ✓<br>(Compulsory, case by case evaluation) |
| | **Food** | 20 | | | | ✓<br>(Compulsory, case by case evaluation) |
| | **Pesticides** | 21 | | | | |
| **United States** | **Cosmetics** | 22, 23 | ✓ | ✓*<br>(Compulsory) | ✓ | ✓<br>(Voluntary)<br>(Compulsory post-market evaluation but no specification for ENMs) |
| | **Drug** | 24 | | | | ✓*<br>(Compulsory) |
| | **Food** | 22, 25, 26 | ✓ | ✓*<br>(Compulsory) | ✓ | ✓*<br>(Compulsory) |
| | **Pesticides** | 27 | | | | ✓*<br>(Compulsory) |

being produced or imported at volume smaller than 10 tons/year (EPA 2014). However, influences of ENMs might be equivalent to or greater than their bulk materials even if they are at smaller production volume due to their increased reactivity and toxicity with enlarged surface-to-volume ratio (Beaudrie et al. 2013). Consequently, such exemptions might have neglected potential risks of ENMs.

## Challenges faced by the regulators

Deficiencies in regulations of ENMs are, in part, due to uncertainties and extensive gaps in knowledge, such that accurate assessments of ENMs have been limited (Bernhardt et al. 2010; Linkov et al. 2009; SCENIHR 2009). Conventional frameworks for assessments of risks contain two major components, i.e. evaluation of hazard and exposure. Evaluation of hazard is hindered by uncertainties resulting from lack of standardized test protocols (Beaudrie et al. 2013; Grieger et al. 2010; Hansen and Baun 2012). Diverse physicochemical characteristics of ENMs that might affect toxic potency, further complicate the issue (Beaudrie et al. 2013; Beaudrie and Kandlikar 2011; Grieger et al. 2010). Consequently, there is a lack of knowledge of toxic mechanisms of ENMs (Beaudrie and Kandlikar 2011). Concurrently, limited disclosure of information about contents of products containing ENMs results in insufficient exposure data for ENMs (Beaudrie et al. 2013; Grieger et al. 2010; Hansen and Baun 2012). Reliable methods for characterization and quantification of ENMs in complicated environmental matrices are also limited (Hansen et al. 2008b; Linkov and Satterstrom 2008; Linkov et al. 2009). Furthermore, with wide applications, ENMs are released through various sources and therefore, assessment of their environmental fates, such as their transformation and bioaccumulation, is difficult (Beaudrie and Kandlikar 2011; Hansen et al. 2008a; Linkov and Satterstrom 2008).

Different reviews in the past have only focused on part of the problems related to the development of regulations on nanomaterials. Therefore, based on the literature, we have identified five challenges faced by regulators in formulating regulations: (1) Nanomaterials exhibit variable physicochemical characteristics, which make it difficult for regulators to establish widely accepted criteria for regulatory definition; (2) There is insufficient knowledge to monitor diverse sources and pathways and to predict fates of ENMs; (3) No method is currently available to reliably quantify exposure of ENMs at small concentrations in complicated environmental matrices; (4) It is challenging to evaluate bioavailability of ENMs, especially after their transformation in the environment; and (5) There is a lack of understanding of their toxic mechanisms. In following sub-sections, further explanations on these five limitations are made with support from various examples.

### Difficulties in defining ENMs

To establish guidelines and regulations for testing of materials, definitions and their corresponding scope are important. Although several regulation unions have tried to establish standards, there is still no globally accepted definition for 'nanomaterial'. Current definitions can be classified into three major categories.

First, ENMs are generally defined according to a threshold of size. The International Organization for Standardization (ISO) defines ENMs as materials with at least one dimension smaller than 100 nm, while engineered nanoparticles are materials with all dimensions smaller than 100 nm (ISO 2015). The Organization for Economic Co-operation and Development (OECD), the European Commission and Canada, etc. adopt similar definitions. Second, some jurisdictions differentiate ENMs by emphasizing their nano-specific properties: The United States Environmental Protection Agency (U.S. EPA) defines ENMs as materials that have unique properties, relative to the same chemical substance in larger sizes. A third classification is a combination of both size and property, such as the one adopted by Australia.

Differentiation of ENMs and their bulk counterparts by particle size is a relatively simpler and clearer way that regulators and manufacturers might more easily follow. Size is also the major intrinsic characteristic for ENMs to stand out from their bulk counterparts (Nel et al. 2006). As a classic example, gold (Au) bulk particle is a noble metal while nanoclusters formed by Au atoms can have catalytic properties (Gruene et al. 2008). Nevertheless, small and bulk are relative terms to describe size of particles. For monitoring exposure and determining potential to cause adverse effects, it is important to determine the critical size of ENMs that leads to change of physicochemical characteristics and hence alters their biological effects (Maynard 2011). However, particle-size dependent toxicity has been suggested to vary between different materials (Karlsson et al. 2009). A comparison of toxicity of silver (Ag) particles ranged from 20 to 113 nm showed that Ag particles at 113 nm can have smaller, similar and larger toxicological effects compared with smaller particles, depending on different endpoints (Park et al. 2011). Such variation made the threshold of 100 nm to be questionable. Concurrently, use of a specific class of physicochemical characteristics to define ENMs is also limited and has been criticized. It has been argued that there is no single most appropriate property for differentiation between ENMs and their bulk counterparts (Handy et al. 2008; Darlington et al. 2009; Bernhardt et al. 2010; Maynard 2011). The large variety of physicochemical characteristics has also triggered concerns of reliable method to measure and categorize ENMs (Fig. 1a, Gottschalk et al. 2013).

These uncertainties have made development of a regulatory definition very difficult. A definition in broad scope includes non-target materials and might increase the cost of regulation while a conservative definition might not accurately assess target materials into proper categories, such that humans and environments are exposed to hazardous materials. This is referred to the regulator's dilemma (Weinberg 1985). Without support from concrete and scientific evidence, different regulating authorities currently adopt different regulatory definitions, which are confusing and makes it difficult for manufacturers and customers to follow.

## Difficulties in tracing possible sources and transport pathways of ENMs

ENMs are used in a broad spectrum of industrial and commercial processes and products and thus can have diverse releasing pathways throughout their product life cycles. These sources can be classified as point and non-point sources, which pose different challenges for regulators. Point sources include effluents and fume gas from factories during manufacture of ENMs and incineration plants during treatment of waste. Various commercial products containing ENMs such as pharmaceuticals, textile coatings and paints can be released to domestic sewage in brown and grey waters **(**Fig. 1b**)**. ENMs from these point sources could be collected through air filtration systems and sewage systems, allowing possibility of removal, monitoring and regulations. For example, ENMs collected by waste water treatment plant (WWTP) could settle or be sequestered into sewage sludge and eventually discarded in landfills (OECD 2015; Tourinho et al. 2012). Consequently, efficiency of removal of ENMs by WWTPs may be determined and regulations may be set for disposal of potentially harmful sewage sludge containing ENMs. Conversely, surface coatings that minimize agglomeration has been proposed to prevent ENMs (cerium oxide ($CeO_2$) NPs) to settle down and remove from effluents of biological-based WWTPs (Limbach et al. 2008). Furthermore, non-point sources such as accidental spills during transportation, car pipe exhaust, dissipation from antimicrobial spray and sunscreen products can result in direct and multi-point discharges into the environment **(**Fig. 1c**)**. For example, ENMs in sunscreens and skin whitening products (e.g. NPs of titanium dioxide ($TiO_2$) and ZnO) can be readily released into aquatic environments from skin of sunbathers who apply these products. These non-point sources, together with ENMs that escape from the collection and removal systems, make regulation of ENMs through sources of release more difficult. This is because once these ENMs are released into the environment, they will be transported to various environmental compartments, making them hard to trace. Aquatic environments and sediments are regarded as sinks where airborne ENMs are deposited, ENMs from terrestrial discharges concentrate and aggregated ENMs settle **(**Fig. 1e**)**. For example, industrial and domestic discharges are attributed as major sources of metal-based ENMs to the environment (Gottschalk and Nowack 2011). The most vulnerable areas should, therefore, be coastal areas and estuaries that receive most of the terrestrial discharges.

Situations can become more complicated when both physicochemical characteristics of ENMs and environmental conditions can govern agglomeration and aggregation of ENMs and therefore their transport in these environmental compartments **(**Fig. 1e**)**. For instance, surface coatings of ENMs and stabilizing surfactants which are common agents for manufacturers to restrict agglomeration of ENMs, can prevent settling of ENMs in the aqueous environment (Hotze et al. 2010; Limbach et al. 2008). With smaller sizes of agglomeration, ENMs (aluminium (Al) NPs with negative coatings of phosphates) can also be transported through the soil column more easily (Darlington et al. 2009). Similarly, various characteristics of seawater and soil can affect transport of ENMs. Natural organic matter, compared to industrial surfactants, has greater abilities to stabilize multi-walled carbon nanotubes (MWCNT) (Kennedy et al. 2008). Greater ionic strength of seawater favours agglomeration and aggregation of ENMs, while stratification and turbulent motion in ocean can retain them in upper layers (Harrison et al. 2003; Keller et al. 2010). Clay loam soils that have larger surface areas can chelate with $Fe^0$ NPs and hence reduce their mobility (El-Temsah and Joner 2012).

Concomitantly, some studies have proposed that certain ENMs could be transferred through food chains. This raises concern that discharged ENMs might be accumulated into bodies of humans via food chain transfer (Beaudrie and Kandlikar 2011). For example, laboratory studies have shown that silver-containing ENMs (polyvinylpyrrolidone-coated Ag nanowires and Ag NPs respectively) could be transferred via both freshwater and terrestrial food chains (Chae and An 2016; Kwak and An 2016). Dietary intake of $TiO_2$ NPs of the zebrafish (*D. rerio*) from their prey at lower trophic level could contribute more to the body burden than from aqueous exposure alone (Zhu et al. 2010). However, all previous studies were conducted under laboratory conditions and along simplified food chains without providing evidence-based evaluation of the situation in the natural environment or determining the bio-magnification factor of the ENMs of concern.

With such diverse sources, transport pathways and potential influential factors, it is difficult to predict depositions of ENMs once they enter the environment. Influences of ENMs might even increase with their potential transport through food chain. Insufficient knowledge in transport and fate of ENMs limits the ability of regulators to evaluate exposure of ENMs during assessment of risk of ENMs. Thus, regulators might not be able to identify potential vulnerable areas and organisms for focus of regulation and protection.

## Difficulties in quantifying ENMs in environmental matrices

Assessment of exposure for regulations of ENMs becomes more difficult due to a general lack of reliable techniques to quantify ENMs after they are released into the environment where they can interact with organic and inorganic constituents of the environment. Besides the traditional labour-intensive methods such as filtration and microscopy (Johnson et al. 2011; Neal et al. 2011; Westerhoff et al. 2011), newer techniques are being developed but all of them need to be improved and validated before broadly applied for environmental analyses. Cloud point extraction (CPE) by surfactants Triton X-114 has been used to extract ENMs from surface water and sewage (Hartmann and Schuster 2013; Liu et al. 2009; Majedi et al. 2012). CPE has high selectivity between ionic and colloidal ENMs, and a small detection limit at level of 0.01–0.001 μg/L could be achieved under optimized extraction conditions in these studies. Nevertheless, no ENMs could be found in collected environmental samples and a large variation in recovery (~50–130%) has been observed in spiked environmental sample, indicating potential low concentration of ENMs that out of detection limit and interference of environmental matrices. Also, classification of sizes of ENMs still depends on intensive microscopic examination. Single particle-ICP-MS (SP-ICP-MS) is useful for determining particle sizes of ENMs and has a detection limit up to nanogram level in unspiked environmental samples (Mitrano et al. 2012b; Tuoriniemi et al. 2012). However, SP-ICP-MS has poorer selectivity towards ENMs with great dissolution and is limited for simultaneous detection of multiple elements (von der Kammer et al. 2012). Pre-treatment of samples such as ion exchange resin to remove dissolved ions might be required to improve its applicability (Hadioui et al. 2015). Asymmetric flow field flow fractionation (AF4) is another method to distinguish particles of different sizes. It has a better resolution to discriminate sizes than does SP-ICP-MS, but it heavily relies on preparation of samples to minimize influences from other matrix constituents (Mitrano et al. 2012a; von der Kammer et al. 2011). Consequently, neither of these methods have been adopted in real applications and it remains a great difficulty to distinguish ENMs from their bulk counterparts, when ENMs present at relatively small concentrations and in the presence of complicated environmental matrices (Gottschalk et al. 2013).

Alternatively, some studies have relied on modelling to predict exposures (Gottschalk et al. 2013). These models use various parameters such as production volume, concentrations of ENMs in commercial products and potentials for release of ENMs to the environment, to estimate environmental concentrations of ENMs (Gottschalk et al. 2011; Gottschalk et al. 2009; Mueller and Nowack 2008; Sun et al. 2014). However, modelling methods have inherent uncertainties, which are evidenced by large variations in their predictions (**Online Resource Fig. S1**, Gottschalk et al. (2013)). The difference between realistic and high exposure scenarios can be as much as 10-fold when these two scenarios have been used to address uncertainties of data collected (Mueller and Nowack 2008). This is not only because the data used by these models are not reliable, but also because the models themselves contain insufficient detail to allow for better resolution. For instance, estimation of production volume depends on data from companies while accurate data are not readily available due to business privacy against disclosure of relevant information (Beaudrie and Kandlikar 2011; Hansen and Baun 2012). During the survey of companies producing and using ENMs, rates of response by the companies were small (Piccinno et al. 2012; Schmid and Riediker 2008). A similar method even failed in obtaining any information (Hendren et al. 2011).

An alternative method that requires more effort has, therefore, been used to collect information from various sources including government and company websites, patents and then estimated production volumes indirectly based on production capacity and product types (Hendren et al. 2011). However, this alternative also has uncertainties. According to one of the most established inventories, 71% of recorded commercial products do not provide sufficient information to corroborate their application of ENMs in the products and most data come from sources that might have not been properly verified with reliable techniques (Beaudrie and Kandlikar 2011; Hansen et al. 2008b; Vance et al. 2015). Furthermore, ENMs in some products of the inventory have only been listed with general names such as 'organics' and 'ceramics', which provide little information to estimate either exposure or hazard potencies of corresponding ENMs **(Online Resource Fig. S2)**. These uncertainties could be attributed to lack of regulations such as labelling and registration mechanisms to provide relevant information. Enforcement of laws is also important: the U.S. EPA has issued a voluntary reporting mechanism (Nanomaterial Stewardship Program) calling for reports from companies involved in production and use of ENMs, but only 16 companies responded to this call, of which only 2 included their production capacity (Hendren et al. 2011). Similarly, the Voluntary Reporting Scheme for Manufactured Nanomaterials issued in the United Kingdom received only 11 responses from industry (Defra 2009).

More fundamentally and might be more lethally, quantification of ENMs simply by mass concentrations might not be suitable for ENMs (Hristozov et al. 2012; von der Kammer et al. 2012). This is because influences of ENMs might be greater than their bulk materials even if they are at similar level of exposure due to their increased reactivity and toxicity with enlarged surface-to-volume ratio (Beaudrie et al. 2013). Physicochemical characteristics of ENMs, such as specific surface area, particle number and distribution of size of pristine or aggregated particles, have been proposed as more relevant parameters for assessment of exposure in estimating risks of ENMs (Hoecke et al. 2009; Oberdörster et al. 2007).

Reliable techniques to quantify ENMs are prerequisite to answer fundamental questions related to environmental concentration, temporal and spatial transport that are necessary for evaluation of exposures in the assessment of their environmental risks. Evaluation of toxicities at environmentally relevant concentrations is also critical for accurate estimation of hazard. Without such information, it is difficult for regulators to determine potential risk of ENMs. Further, validated and accurate methods to quantify ENMs in the environment are also necessary for evaluation and enforcement of proposed environmental quality objectives in regulations.

## Difficulties in evaluating bioavailability of ENMs

In traditional chemical-based frameworks for assessment of hazards, bioavailability of chemicals must be evaluated and dissolved portions of chemicals have been regarded as bioavailable fractions of contaminants (Di Toro et al., 1991). However, this conventional concept might not address the full problem of ENMs (Hristozov et al. 2012; Metcalfee et al. 2009).

Dissolved portions of ENMs has been proposed to follow the definition of colloidal particles, i.e. the fraction that can pass through a 0.45 μm filter and includes both dissolved and colloid species (Klain et al. 2008). However, in the environment, ENMs that aggregate to larger size (> 0.45 μm) might also be bioavailable to biota. For instance, aggregation of particles might increase their potential of exposure to filter feeders, which filter a large volume of water with their filtering cilia to catch particles for food (Baun et al. 2008). Cladoceran species, a common group of filter feeders, feed on particles with a size range of 0.4–5 μm (Geller and Müller 1981; Gophen and Geller 1984), which is generally larger than above threshold value (0.45 μm). Similarly, both suspension- and deposition- feeding mussels can capture particles at sizes between 0.7–150 μm (Sprung and Rose 1988; Taghon 1982). Moreover, intake of ENMs in large size does not mean that organisms are safe from ENMs since agglomerations of ENMs joined by weak forces might disassociate to smaller particles subjected to changes in environmental conditions.

Transport of ENMs can be governed by their physicochemical characteristics and environmental conditions, while the final location for deposition of ENMs determines their availability to different groups of organisms (**Fig.** 1f**)**. Acting against the tendency of ENMs to agglomerate and aggregate, surface coatings and stabilizing surfactants of ENMs, as well as stratification and turbulent motion in oceans can retain ENMs in upper layers (Darlington et al. 2009; Hotze et al. 2010; Kennedy et al. 2008) and consequently reduce exposure of sediment-dwelling organisms to ENMs while increase exposures of pelagic species (Harrison et al. 2003; Keller et al. 2010). Similarly, chelation to organic matter and inorganic ligands can reduce availability of ENMs to organisms (Tong et al. 2007; Tong et al. 2012). Furthermore, although some ENMs are functionalized with different coatings during manufacture, coatings might be partially or fully degraded by light, oxidants and microorganisms in the environment, resulting in exposure of altered ENMs rather than the ENMs with original coatings (Auffan et al. 2010; Nowack and Bucheli 2007).

Some ENMs, such as those used in environmental remediation of contaminated sites, are designed with large surface areas and high reactivity to promote interactions with other compounds (Chen et al. 2009; Jarošová et al. 2015; Zhao et al. 2015). When these ENMs are released into the environment, their interaction with other environmental pollutants can result in alterations of bioavailability and toxicity of both the ENMs and adsorbed pollutants **(**Fig. 1f**)**. Carbon nanotube (CNT), SWCNT and MWCNT have strong affinities for aromatic compounds with nitro-functional groups, non-polar aromatic and non-polar aliphatic compounds which are used for insecticide and dye synthesis (Chen et al. 2007). Strong affinity of these compounds with CNT might reduce their bioavailability (Nowack and Bucheli 2007). Alternatively, attachment of pyrene, phenanthrene and naphthalene with CNT can be reversible (Yang and Xing 2007), which might result in desorption of these toxic compounds after ingestion by animals. Similarly, $As^{5+}$ ions that adhere to $TiO_2$ NP could be ingested by the common carp, *Cyprinus carpio* and such adsorption has facilitated the accumulation rate of $As^{5+}$ ions and nearly doubled its accumulation amount (Sun et al. 2006).

Determination of bioavailability of ENMs is the initial step for evaluation of hazards of ENMs because bioavailable forms of ENMs can cause direct biological influences towards organisms and even accumulate along the food chain. However, more studies are required, given the complicated interactions between ENMs and their physicochemical characteristics, environmental factors, as well as interaction with other pollutants before their potential hazards can be properly assessed for regulators.

## Difficulties in interpreting toxicity of ENMs

Relatively more information is available for assessments of hazards of ENMs compared with assessments of exposure. However, more issues need to be addressed to have a comprehensive assessment of hazards of ENMs **(**Fig. 1g**)**.

A continuous argument in this area is whether there is a difference of toxicity between ENMs and their bulk counterparts because ENMs usually aggregate in the environment. Current studies suggest that the size-dependent toxicity can be varied with different ENMs and test species (Wong et al.

2013; Yung et al. 2015b). For instance, ZnO NPs could become more toxic even though they have similar or larger aggregate size as their bulk particles in suspension towards the algae *Chlorella* sp. (Ji et al. 2011), *S. costatum* and *T. pseudonana* (Wong et al. 2010), as well as the crustacean *D. magna* (Heinlaan et al. 2008). Contrarily, studies suggest that ZnO NPs have similar or less toxicity than bulk ZnO towards the alga *P. subcapitata* (Aruoja et al. 2009; Franklin et al. 2007), the crustaceans *T. japonicus* and *E. rapax* (Wong et al. 2010) and the zebrafish *D. rerio* (Xiong et al. 2011). Similar controversies are also found for CNTs. Some studies suggested CNTs with shorter length and/or smaller diameter can adhere to model organisms more easily and hence have larger toxicity (Cheng et al. 2007; Kennedy et al. 2008), while other studies found that there is no size-dependent toxicity (Li and Huang 2011), or the size-dependent toxicity can vary among species (Mwangi et al. 2012).

Within same categories of ENMs, such as metal-based ENMs, uncertainties remain in interpreting their primary mode of toxic action. Study of Ag NPs on the common grass *Lolium multiflorum* demonstrate that toxicity could not be attributed solely to ions in solution because its toxicity could not be reproduced by the same amount of $Ag^+$ ions generated from $AgNO_3$ (Yin et al. 2011). The bacteria, *Cupriavidus necator* and *Escherichia coli* have exhibited different molecular responses when they are exposed to ZnO NPs or $Zn^{2+}$ ions, with ZnO NPs interfering with cell membrane proteins and synthesis processing of functional gene products (Neal et al. 2012; Su et al. 2015). Other studies on NPs of $CeO_2$, ZnO and $TiO_2$ also suggest that production of reactive oxygen species (ROS) from nanoparticles besides dissolution of ions could contribute to toxicities of ENMs (Li et al. 2012; Xia et al. 2008). Conversely, it has been argued the difference might be due to the chelation with medium matrices that rates of ion dissolution from ENMs (Ag NPs) are under-estimated (Xiu et al. 2012). Some studies also suggest that dissolved ions were the cause of most of the observed toxicities of NPs of Ag, CuO and ZnO (Blinova et al. 2010; Franklin et al. 2007; Wong et al. 2010; Xiu et al. 2012).

The issue has become more complicated when ENMs can be easily modified during manufacturing processes. For instance, ENMs of similar core compositions, can have different shapes, crystalline structures, functionalities and product matrices or surfactants into which they are incorporated **(**Fig. 1a**)**. These properties have been shown to be correlated with influences of ENMs on biota. Shape of ENMs could affect their internalization by different bacterial and mammalian cell lines and hence their corresponding toxicity (Cardillo et al. 2016; Forest et al. 2017; Simon-Deckers et al. 2009). Variation in crystalline structure and crystal facets of ENMs could have different surface energies to produce ROS and release ions (De Matteis et al. 2016; Fan et al. 2012; Liu et al. 2016). ZnO NPs coated with different silane chains could have different toxic potencies towards bacteria, varied as a function of modified capability to release $Zn^{+2}$ ions and produce ROS (Hsu et al. 2014; Leung et al. 2012). Surfactants such as sodium dodecyl sulphate (SDS) and polyoxyethylenesorbitane monooleate (Tween 80) have been suggested to reduce aggregation and enhance bactericidal activities of Ag NPs (Kvitek et al. 2008). Meanwhile, same surface coating could cause different changes in toxicity to bacteria for ZnO NPs from different supplying brands (Hsu et al. 2014).

Besides physicochemical characteristics of ENMs, transformation of ENMs due to various environmental factors and other pollutants also complicates the issue since these transformed ENMs could have very different biological influences compared to their pristine forms (Wong et al. 2013; Yung et al. 2015b). For example, in aquatic systems, both temperature and salinity could alter sizes of particulates and dissolution of ions and thus toxicity of ZnO NPs to marine organisms (Wong and Leung 2014; Yung et al. 2015a, 2017). Natural organic matters could also decrease toxicity of MWCNT towards the freshwater water flea, *Ceriodaphnia dubia*, due to its reduced physical damage with smaller size of aggregates and lesser affinity to the lipid membrane of intestinal cells (Kennedy et al. 2008; Tang et al. 2016). In soils, chelation to organic matter and inorganic ligands could reduce toxicity of fullerene and single-walled CNT due to reduction of their bioavailability (El-Temsah and Joner 2012; Tong et al. 2007; Tong et al. 2012). However, effects of surface-bound humic acid on toxicity of $TiO_2$ NPs could be dependent on their crystal structure, resulting in increased toxicity for anatase and decreased toxicity for rutile (He et al. 2016). Furthermore, mixtures of $TiO_2$ with different crystalline structures could have either addictive or antagonistic effects compared to the toxicity of individual crystalline structure, depending on concentrations of rutile and anatase in the mixture (Iswarya et al. 2015; Iswarya et al. 2016). Similarly, a mixture of Ag, copper (Cu) and silica ($SiO_2$) NPs could be more toxic than the summation of toxicities of individual constituent ENMs to the Artic microbial community even when they are at threefold lesser concentration (Kumar et al. 2012).

Moreover, different test methods such as dispersion methods for ENMs to suspend in aqueous medium during the toxicity test can result in different particle size distributions and influence the toxicity (Hartmann et al. 2015). For instance, sonication has resulted in smaller median aggregation size of double-walled CNTs and hence larger toxicity when compared with simple mechanical mixing by stirring (Kwok et al. 2010). Therefore, there would be a chance of overestimation of the toxicity and environmental risk of ENMs if sonication or solvent, which are less environmentally relevant, were used as the dispersion methods.

There are still many controversies for toxicity and mode of action of ENMs, especially under influences of various physicochemical characteristics of ENMs, different environmental factors and with a lack of standardized protocols for test of toxicity. Without understanding of potential toxic mechanisms and toxicity of ENMs after various industrial modifications and environmental conditions, regulators could not establish comprehensive assessment of hazard of ENMs. Failure in identification of possible toxic mechanisms and endpoints can also prevent vulnerable groups from special protection.

## Suggestions for future regulations

Large uncertainties and extensive knowledge gaps make assessment of risk for ENMs very challenging. Compounded by rapid development of nanotechnology, it is almost impossible to adopt "one-for-ever or one-for-all" regulations for ENMs. One approach is to initiate easy-to-achieve, progressive guidelines to regulate ENMs based on current knowledge, and then continually update and adapt the regulations based on knowledge as it is gained (Beaudrie et al. 2013; Grieger et al. 2010; Hansen et al. 2008a; Linkov et al. 2009). Therefore, in following sections, methods to concentrate research effort and prompt regulations that facilitate assessment of risks of ENMs are proposed. Besides these qualitative methods, emerging tools that assist in quantitative regulations based on assessment framework of risk and decision making of regulators are also suggested.

### Prioritizing ENMs for regulations

It is challenging to address potential risks of ENMs with such dramatic growth and extensive application of ENMs. Therefore, a prioritized ranking which lists ENMs with greater risk should be proposed to call for immediate attention and research to support early development of their regulations (Grieger et al. 2010; OECD 2010). The ranking proposed is developed with consideration of the two components, evaluations of exposure and hazard, in a general framework for assessment of risk. Here, relative exposures are estimated, based on volumes of production and usage in commercial products **(Online Resource** Figs. S1 and S2**)**, while hazard is determined from laboratory tests of toxicity to individual species **(Online Resource** Fig. S3**)**. Based on the information **(Online Resource Section 2)**, it is proposed that ENMs requiring immediate research to support regulations are: NPs of Ag, ZnO, $Fe_2O_3$ and $TiO_2$. Importantly, a large portion of the identified commercial products that contain Ag, ZnO and $TiO_2$ NPs are sprays, cleaning detergents and personal care products (e.g. sunscreens and cosmetics), which might easily release their constitutional ENMs during usage **(Online Resource** Fig. S2**).** This further highlights that these ENMs are of potentially high risks (Hansen et al. 2009; Hansen et al. 2008a). A similar but more extensive list of ENMs has been proposed by OECD to act as representatives for understanding of measurement, toxicology and risk assessment of other ENMs (OECD 2010).

Relative amounts of research on ENMs have been compared based on numbers of research articles available on the search engine Web of Science **(Online Resource** Fig. S4**)**. Fewer studies have been conducted on the other two ENMs ($Fe_2O_3$ and ZnO NPs) that are also focuses of the present review. Therefore, future research might require more effort on the remaining two ENMs. Major areas of research should be development of techniques for quantification, evaluation of their bioavailability under different environmental conditions, and study of their toxic mechanisms towards different species. Moreover, these lists should only represent temporary prioritization because all data come from available peer-reviewed articles, which are subjected to limitations mentioned above. Therefore, upon improvement of knowledge, these lists should be updated.

### Methods to promote conventional assessment of risk of ENMs

To achieve comprehensive assessments of risk of ENMs, many knowledge gaps have to be filled but some of them might be addressed promptly by administrative means of authorities. In current practices, a pre-market evaluation is usually initiated with the pre-manufacture notice (PMN) or significant new use notice (SNUN) from manufactures when they have new commercial products. Output from the evaluation will determine whether a permit can be issued for the product to be on sale in the market **(**Fig. 2**, arrows in black)**. According to the conventional framework of risk assessment, pre-market evaluation should involve evaluations of exposure and hazard of the ENM used in the proposed product.

Given limited reliable techniques for direct quantification of ENMs, predictive models that estimate production volume and environmental concentrations of ENMs might be a better method currently available for evaluation of exposure of ENMs. Current predictive models are suffering from limited and unreliable information due to business privacy and lack of guidelines for disclosing relevant information, but these uncertainties of predictive model might be relieved when regulative authorities step in to facilitate disclosure of information (Brown 2009; Hansen et al. 2008b). For example, a compulsory reporting scheme on ingredients of commercial products by manufacturers could be mandated to gather information for predictive models to estimate exposures of ENMs **(**Fig. 2**, arrows in red)**. Information to be reported could include type, physicochemical characteristics, concentrations of ENMs used in the products and their possible life cycle. Information provided might further populate a database for scientists and relevant authorities to estimate potential exposure to ENMs, which is an important component in the conventional assessment framework of risk **(**Fig. 2, **arrows in**

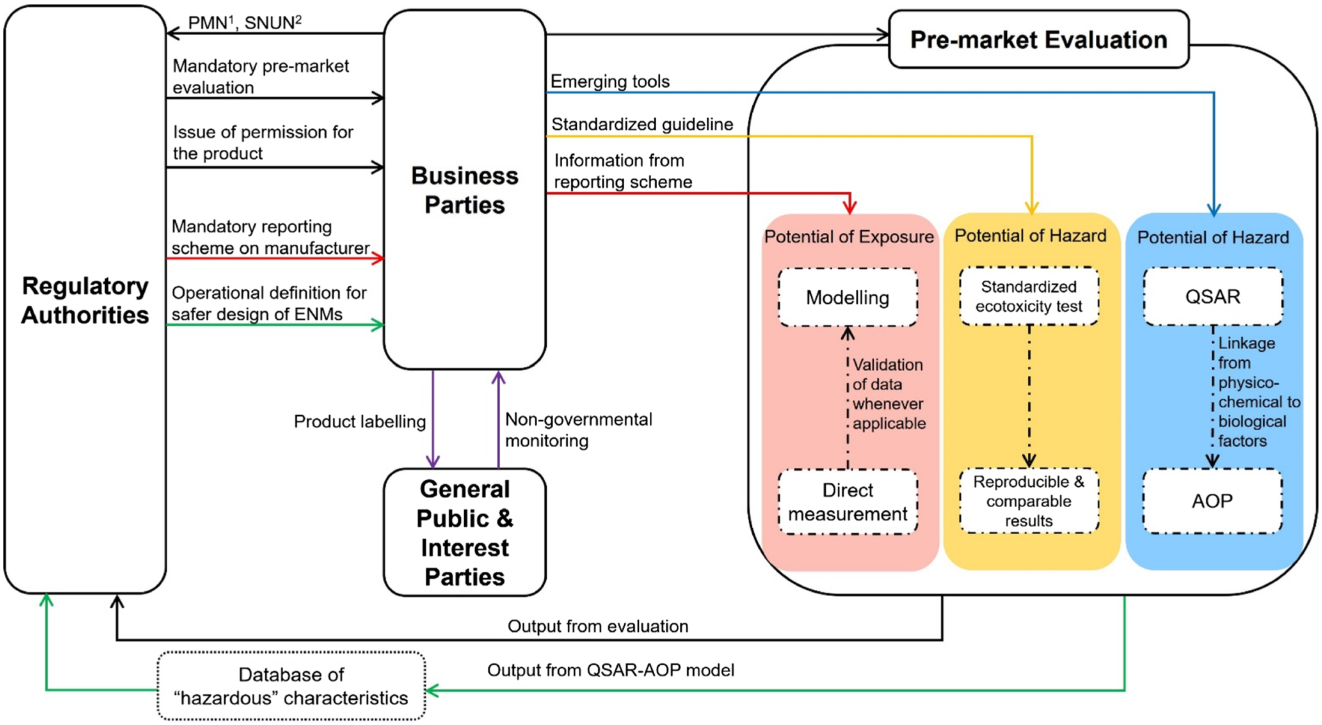

**Fig. 2** Conventional and modified risk assessment framework engineered nanomaterials. (1) PMN: Pre-manufacture notice, and (2) SNUN: Significant new use notice

**red)**. Moreover, validation of environmental concentrations of ENMs should be performed by cross-checking between results from predictive models and emerging quantifying techniques whenever they are developed **(**Fig. 2**, box in red)**. More precise and accurate data from assessment of exposure allow authorities to review the necessity to regulate ENMs and thus could serve as an early-warning system for environmental managers to proactively mitigate risks of ENMs. Moreover, compulsory labelling schemes that specify ENMs should also be mandated so that customers and other interested parties can serve as public monitoring ambassadors on manufacturers (Fig. 2, **arrows in purple**; Linkov et al. 2009; Malloy 2011).

Once continuous information on production volume becomes available for ENMs, the next stage is to foster a mandatory pre-market evaluation before commercial products containing ENMs enter the market. This comes to another component of assessment of risk, the evaluation of hazard of ENMs. To generate reproducible and comparable results, it is important for regulators to develop standardized protocols to determine effects and potencies of ENMs (Fig. 2**, arrows and box in yellow**; Hartmann et al. 2015; Linkov et al. 2009). Efforts have already been addressed by some parties and now toxicity test guidelines have been developed and reviewed for their adequacy; Existing Test Guideline 102, 109, 113, 116 were proposed as valid for ENMs (OECD 2009). As a mean to improve toxicity testing of ENMs, a series of guidelines on preparation of samples, dosimetry and ecotoxicity have also been reviewed by OECD (OECD 2012; OECD 2014). OECD concluded that current guidance for testing bulk chemicals could be applied to ENMs, though some adaptations would be necessary. Some modifications of protocols, for example, methods to maintain constant exposures of ENMs to model organisms in aqueous media and potential endpoints for measurement, have been suggested (Hartmann et al. 2015; Hund-Rinke et al. 2016). Moreover, ENMs are prone to different physicochemical characteristics and environmental factors. Therefore, during assessment of hazards of ENMs, it is important for the guidance to consider the influences of these parameters, as well as testing at environmentally relevant concentrations, which could be estimated based on information from above compulsory reporting scheme.

In summary, with more comprehensive information from the compulsory reporting scheme, evaluation of exposure of ENMs could be improved. The standardized guidance addressing different influential factors could also produce comparable and realistic results for manufacturers and regulatory agencies during their assessment of hazard for products containing ENMs. Furthermore, standardized methodologies allow joint efforts from various research parties and thus reduce time and resources to deal with risk assessment of ENMs. During pre-market evaluation and development of regulations by authorities, results from these two assessments could then be compared to estimate risk of ENMs. However, in such traditional regulatory framework, three out of the five proposed limitations, i.e. difficulties in defining ENMs, tracing their transport pathways and understanding of their toxic mechanisms could not be addressed. To better address the assessment of risk would also necessitate development of probabilities of exceeding thresholds for effects to a defined proportion of individuals or a specified proportion of species. Furthermore, rapid development of nanotechnology makes it very challenging to investigate each product containing ENM as soon as it is newly produced.

## Emerging tools to facilitate conventional assessment of risk of ENMs

The conventional assessment framework of risk that requires solid support of information might not be suitable due to broadening gaps in information under rapid development of ENMs and their expanding uses in products (Beaudrie et al. 2013; Beaudrie and Kandlikar 2011; Hristozov et al. 2012; Linkov et al. 2009). It is, therefore, important to adopt tools that provide simple and high-throughput screening of ENMs before they become available in market.

Adverse outcome pathway (AOP) aims to identify an explanatory pathway linking a molecular initiating event (MIE, chemical-biomolecule interaction) with apical measurement endpoints and estimate possible adverse outcomes at organism or population levels. The AOP construct has large tolerance for plausible and hypothetical linkages, and allows assessments even in the absence of some information. Accordingly, although knowledge on the toxic mechanisms of ENMs is complicated and missing, assessment of hazards of ENMs can still be fitted into AOP because it allows information to be obtained from different sources including in vitro, in vivo and in silico systems (Cote et al. 2016; Juberg et al. 2017; Knudsen et al. 2015; Rowlands et al. 2014). Furthermore, the AOP construct helps to narrow knowledge gaps by identifying missing linkages in the pathway and then future research efforts can be concentrated to fill these knowledge gaps (Ankley et al. 2010).

The AOP concept could also be combined with models that enable linkages between physicochemical characteristics of ENMs and toxicological observations, such as quantitative structure-activity relationship (QSAR) models (Cote et al. 2016; Knudsen et al. 2015; Mu et al. 2016). An integrative QSAR-AOP model enables linkage of biological influences to a general physicochemical characteristic shared by various ENMs. For example, metal oxide nanoparticles that have band energies comparable to the redox potentials of antioxidants and formation of radicals have been found to produce oxidative stress and inflammation in vitro (Burello and Worth 2011a; Burello and Worth 2011b; Zhang et al. 2012). This result has been further linked to the photoactivated toxicity (PAT) AOP to establish a transition from physicochemical characteristics to biological endpoints (Ankley et al. 2010). These concepts make it possible to evaluate the AOP (PAT of metal oxide nanoparticles), based on rapid screening of the physicochemical characteristics of ENMs (band gap) (Knudsen et al. 2015). Furthermore, instead of interpreting toxicities of ENMs through one general ecotoxicity test, QSAR-AOP model narrows the target question to one MIE and correlates the event with specific physicochemical characteristics that can predict such influence. This might minimize the variation caused by the diverse and variable properties of ENMs (Holden et al. 2016). Meanwhile, different AOPs can also link with each other to develop a network and therefore can explain complicated pathways leading to a final response, which is very common in ENMs (Knudsen et al. 2015).

Consequently, the QSAR-AOP construct might manage to explain toxic mechanisms of ENMs. More importantly, it minimizes the effort to measure a larger set of physicochemical characteristics of ENMs and allows high-throughput and standardized measurements to be fed into conventional assessment framework of hazards of ENMs **(**Fig. 2**, arrows and box in green,** Zhang et al. (2012)**)**. Currently, several AOPs have already been proposed and shared in an open source (https://aopwiki.org/). With the database growing, it would benefit modelling of hazard of ENMs, which is currently hindered by many knowledge gaps. However, problems in tracing transport of ENMs remain and more fundamentally, the pre-market evaluation will not work efficiently if there is no universal definition among different regulatory bodies.

## Modifications of conventional assessment framework to address risks of ENMs

Instead of investigating potential criteria that describe ENMs correctly and scientifically, we proposed it might be better to apply risk ranking tools which describe risks of ENMs based on available knowledge to aid in decision making of regulators, given the presence of large uncertainties currently (Beaudrie and Kandlikar 2011; Grieger et al. 2010).

Contrary to the conventional assessment framework of risk that aims to predict toxicity or develop criteria that are protective, the alternatives assessment framework develops relative hazard indices that can be used to identify alternatives to replace potential hazardous chemicals. Decision making processes under this framework integrate both quantitative data and qualitative judgement based on social interest and experience from experts. Consequently, the alternatives assessment framework does not require full understanding of risk of ENMs, which is currently lagging behind the rapid development and commercialization of ENMs (Hjorth et al. 2017a; Linkov et al. 2009). For example, four ENMs (NPs of Ag, ZnO, $Fe_2O_3$ and $TiO_2$) are prioritized in this review because of their larger potentials for exposure and greater hazard. Instead of identifying their risks to set up criteria for protection, the alternatives assessment framework will simply identify possible safer replacements for these four ENMs that does not require full understanding of the four ENMs. However, one disadvantage of the alternatives assessment framework is that it cannot discriminate between the same ENM but varies with different physicochemical properties, which is a common situation for ENMs because they are highly flexible in their design. It is even complicated when ENMs are incorporated into commercial products at different purities (Hjorth et al. 2017a; Leung et al., 2015). This problem might be solved by use of information generated from the QSAR-AOP model. For instance, the above example of band gap-PAT relationship demonstrates how band gap can affect PAT of ENMs. Accordingly, the alternatives assessment framework can then select a safer replacement by discriminating between different band gaps of the same ENM.

The concept of alternatives assessment framework and application of the QSAR-AOP model provides a feedback channel for conventional frameworks for assessments of risks (Fig. 2**, arrows in green)**, which might better address assessment of relative risks of ENMs. For instance, according to the results from the QSAR-AOP construct, physicochemical characteristics of ENMs that might lead to harmful biological influences could be identified. With standardized protocols, joint efforts from various research parties could formulate a database that lists out these physicochemical properties. Then based on the list and other qualitative parameters such as social interest and judgement from experts, regulators may rank the relative hazards of ENMs according to their physicochemical properties, i.e. the concept of the alternatives assessment framework to identify safer ENMs **(**Fig. 2**, arrows in green)**. With such a list of characteristics, process of decision making might be facilitated (Hansen et al. 2008a; Hjorth et al. 2017b).

A possible construct in which to apply such a database might be the Scoring and Ranking Assessment Model (SCRAM) developed to rank persistent, bio-accumulative and toxic substances. The SCRAM model assigns ranking scores based on known characteristics of chemicals (e.g. bioaccumulation factor, half-life, acute toxicity, chronic toxicity and toxic mechanisms) and uncertainty scores if data is not readily available (Snyder et al. 2000). Similarly, based on outputs of the QSAR-AOP model, regulators and manufacturers can rank ENMs based on their physicochemical characteristics such as band energy and toxic mechanisms, e.g. how comparable the band energy of an ENM is to the energy to form radicals and how much PAT contributes to the overall toxicity. The final absolute and uncertainty scores can be used as reference during pre-market evaluations to see if the ENM would pass or fail pre-set thresholds of potential hazard and/or uncertainties.

A further step could be development of an operational definition for ENMs. With information from the feedback channel **(**Fig. 2**, arrows in green)**, an operational definition could be formulated and act as a universal guidance for both regulators and manufacturers to avoid ENMs with these hazardous physicochemical characteristics to be produced, i.e. safer by design (Morose 2010; Nel et al. 2013). For instance, ENMs with hazardous band energy can be easily altered with common industrial practices including by modification of size or functionalization of the surface of ENMs (Burello and Worth 2015).

Pitfalls, yet, exists for such a paradigm. QSAR model for ENMs (nano-QSAR) generally requires larger datasets than do traditional QSAR models to account for uncertainties due to the complexity of ENMs and hence efforts and time are needed to select creditable information to minimize variations (Knudsen et al. 2015; Nel et al. 2013). One of the criteria should be comprehensive characterization of ENMs (Warheit 2008). For example, possible links between band energy and ROS production do not consider the fact that capability of ENMs to produce ROS might be dependent not only on its band energy but also on its morphology and the presence of surface defects (Cardillo et al. 2016). Explaining such complex dependences might require sufficiently thorough characterization of physicochemical characteristics of ENMs to understand their relative contribution or inter-correlation. Furthermore, transport and fate of ENMs, which are important components for assessment of risk, are not considered during the risk-ranking approach using the QSAR-AOP approach (Nel et al. 2013). However, it is also recognized that an advantage of QSAR and AOP models is their adaptability to sources of information. Currently, in vitro, in vivo and in silico information are being produced through different high-throughput techniques (Nel et al. 2013). Modifications to QSARs are also being developed to address their deficiencies (Knudsen et al. 2015). Furthermore, the proposed concept of alternatives assessment addresses risk of ENMs during manufacturing process and no later than the stage of pre-market evaluation. It might ease the great challenges to trace the transport pathways of ENMs after their discharge. Consequently, we proposed our suggestions might be the most available substitution at current stage.

## Conclusions

Regulation of ENMs is currently incomplete in all countries. It is proposed that challenges in regulating ENMs is primarily due to a lack of knowledge in traditional assessments of risk. Current definitions for ENMs might fail or be difficult to address all potentially hazardous ENMs. Various physicochemical characteristics of ENMs and their interactions with environmental factors affect both evaluations of exposure and hazard for ENMs. The lack of methods for quantification of ENMs in environmental samples also limits evaluation of exposure and subsequent assessment of whether environmental quality guidelines are being met. However, it is vital to establish regulations for ENMs given their wide application, increasing production volume and potential hazards.

In order to concentrate research efforts, based on production volume, prevalence in the market and potent toxicity, four ENMs (NPs of Ag, ZnO, $Fe_2O_3$ and $TiO_2$) are suggested for immediate investigation and regulation. Comprehensive and advanced investigations should be put on these four ENMs of relatively greater risks to foster earlier regulation. Recommendations of regulations includes an immediate establishment of a compulsory reporting scheme for registration and labelling of products which requires the manufacturers to provide more information on their ENM-incorporated products such as composition, physiochemical characteristics and production volume for evaluation of exposure of ENMs through predictive models. To provide more information in evaluation of hazard of ENMs, standardized guidance should be developed and progressive acquisition of data with the use of advanced tools, the QSAR-AOP model, are proposed. This model might allow rapid screening, generalization of common physicochemical characteristics shared by different ENMs and tolerate uncertainties and knowledge gaps. A further step is to regulate ENMs through their manufacturing processes and pre-market evaluation of commercial products by developing an operational definition based on outputs from the QSAR-AOP model. This addresses influences of ENMs before their manufacture and application and hence might minimize their release into the environment, which is too challenging for current techniques to trace them. Furthermore, this kind of proactive regulation might be better adapted for rapid development of nanotechnology.

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