Environ Sci Pollut Res (2018) 25:3060–3077
DOI 10.1007/s11356-017-9489-0

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

Racliffe W. S. Lai[1] · **Katie W. Y. Yeung**[1] · **Mana M. N. Yung**[1] · **Aleksandra B. Djurišić**[2] ·
John P. Giesy[1,3,4] · **Kenneth M. Y. Leung**[1,5]

Received: 30 March 2017 / Accepted: 7 June 2017 / Published online: 22 June 2017

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

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (doi:10.1007/s11356-017-9489-0) contains supplementary material, which is available to authorized users.

✉ Kenneth M. Y. Leung
kmyleung@hku.hk

[1] The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China

[2] Department of Physics, The University of Hong Kong, Pokfulam, Hong Kong, China

[3] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada

[4] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China

[5] State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

Introduction

Nanomaterials are materials with one or more dimensions in the nano-scale (1–100 nm; one billionth of a meter; 1×10^{-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

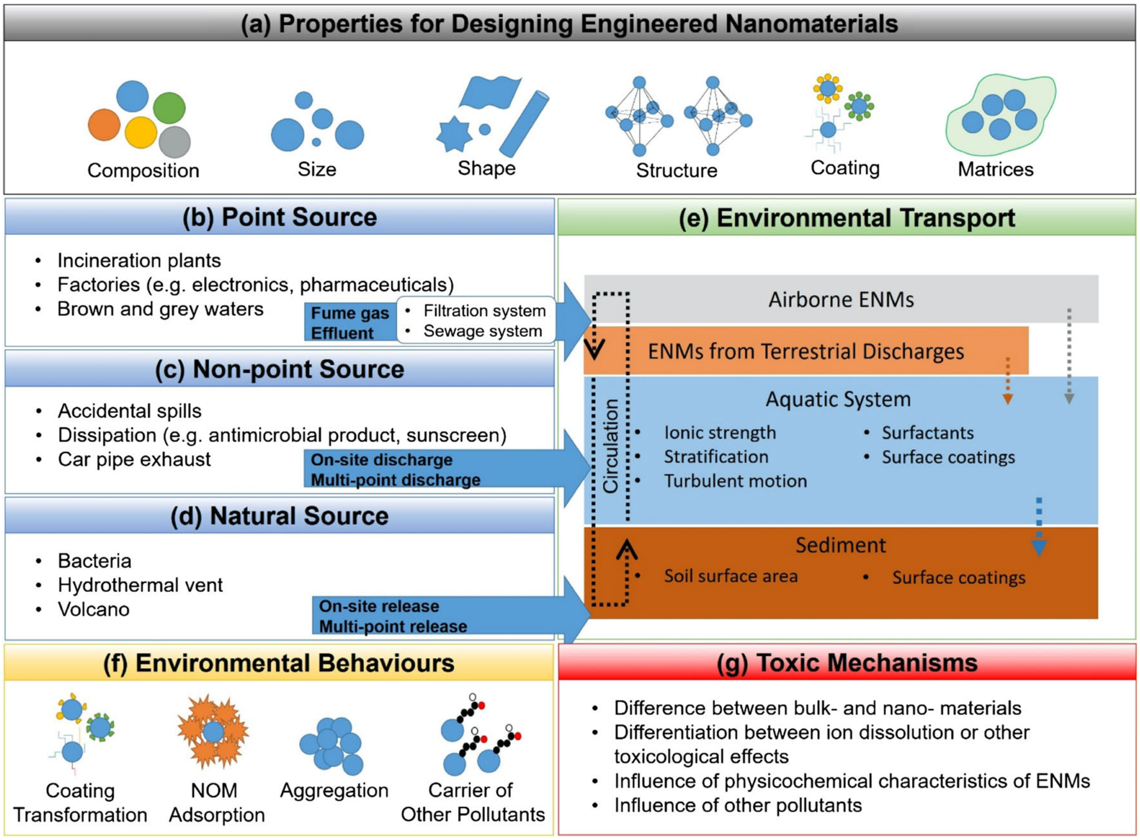

Fig. 1 A schematic diagram summarizing possible environmental behaviours and fates of engineered nanomaterials

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	✓	✓* (Compulsory)		
	Drugs	2				
	Food	3		✓* (Compulsory)		✓* (Compulsory)
	Pesticides	4, 5	✓			✓ (Compulsory)
Canada	**Cosmetics**	6, 7, 8	✓	✓* (Compulsory)		
	Drugs	6, 8, 9	✓	✓* (Compulsory)		
	Food	6, 8, 9	✓	✓* (Compulsory)		
	Pesticides	8, 10, 11	✓	✓* (Compulsory)		✓* (Compulsory)
China						
European Union (including UK)	**Cosmetics**	12, 13	✓	✓ (Compulsory)	✓	✓ (Compulsory)
	Drugs	14, 15	✓	✓* (Compulsory)		✓ (Compulsory)
	Food	16,17	✓	✓* (Compulsory)	✓	
	Pesticides	17, 18, 19	✓		✓	✓* (Compulsory)
Japan	**Cosmetics**	20		✓* (Compulsory)		✓ (Compulsory)
	Drug	20				✓ (Compulsory, case by case evaluation)
	Food	20				✓ (Compulsory, case by case evaluation)
	Pesticides	21				
United States	**Cosmetics**	22, 23	✓	✓* (Compulsory)	✓	✓ (Voluntary) (Compulsory post-market evaluation but no specification for ENMs)
	Drug	24				✓* (Compulsory)
	Food	22, 25, 26	✓	✓* (Compulsory)	✓	✓* (Compulsory)
	Pesticides	27				✓* (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**

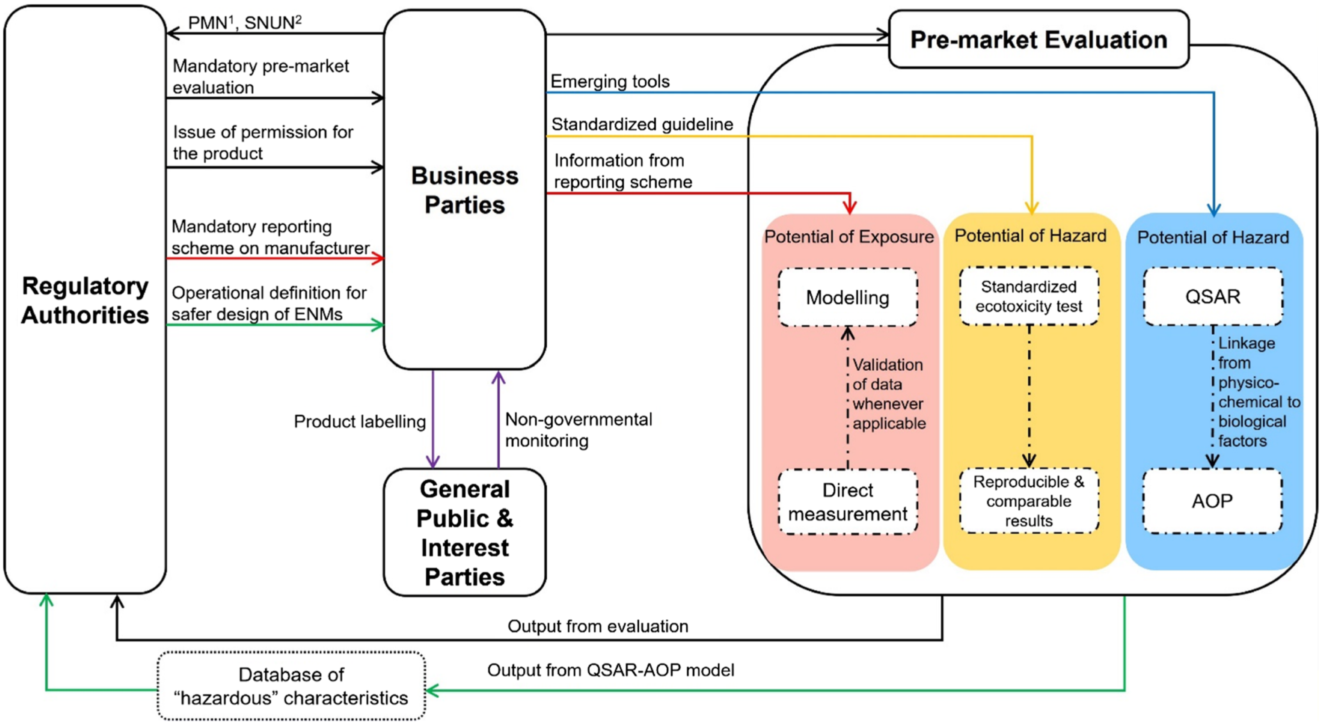

Fig. 2 Conventional and modified risk assessment framework engineered nanomaterials. (1) PMN: Pre-manufacture notice, and (2) SNUN: Significant new use notice

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.

Acknowledgements This project is supported by Research Grants Council of the Hong Kong Special Administrative Region Government via a General Research Fund (no. 17305715), a Collaborative Research Fund (no. C7044-14G) and a Theme-based Research Scheme (no. T21-711/16-R) to KMYL. RWSL thanks the University of Hong Kong (HKU) for partially supporting his Ph.D. study via a Type-B studentship. JPG is grateful to HKU for awarding him the Distinguished Visiting Professorship which enables him to work on this meaningful project.

References

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL (2010) Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29:730–741. doi:10.1002/etc.34

Aruoja V, Dubourguier HC, Kasemets K, Kahru A (2009) Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Sci Total Environ 407:1461–1468. doi:10.1016/j.scitotenv.2008.10.053

Auffan M, Pedeutour M, Rose J, Masion A, Ziarelli F, Borschneck D, Chaneac C, Botta C, Chaurand P, Labille J (2010) Structural degradation at the surface of a TiO_2-based nanomaterial used in cosmetics. Environ Sci Technol 44:2689–2694. doi:10.1021/es903757q

Baun A, Hartmann NB, Grieger K, Kusk KO (2008) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17:387–395. doi:10.1007/s10646-008-0208-y

Beaudrie CEH, Kandlikar M (2011) Horses for courses: risk information and decision making in the regulation of nanomaterials. J Nanopart Res 13:1477–1488. doi:10.1007/s11051-011-0234-1

Beaudrie CE, Kandlikar M, Satterfield T (2013) From cradle-to-grave at the nanoscale: gaps in U.S. regulatory oversight along the nanomaterial life cycle. Environ Sci Technol 47:5524–5534. doi:10.1021/es303591x

Bernhardt ES, Colman BP, Hochella MF, Cardinale BJ, Nisbet RM, Richardson CJ, Yin L (2010) An ecological perspective on nanomaterial impacts in the environment. J Environ Qual 39:1954. doi:10.2134/jeq2009.0479

Blinova I, Ivask A, Heinlaan M, Mortimer M, Kahru A (2010) Ecotoxicity of nanoparticles of CuO and ZnO in natural water. Environ Pollut 158:41–47. doi:10.1016/j.envpol.2009.08.017

Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A (2013) Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells *in vitro*: a critical review. Arch Toxicol 87:1181–1200. doi:10.1007/s00204-013-1079-4

Brazell L (2012) Nanotechnology law: best practices. Wolters Kluwer Law & Business, Kluwer Law International, Alphen aan den Rijn, The Netherlands

Brown S (2009) The new deficit model. Nat Nanotechnol 4:609–611. doi:10.1038/nnano.2009.278

Burello E, Worth AP (2011a) QSAR modeling of nanomaterials. Wiley Interdiscip Rev Nanomed Nanobiotechnol 3:298–306. doi:10.1002/wnan.137

Burello E, Worth AP (2011b) A theoretical framework for predicting the oxidative stress potential of oxide nanoparticles. Nanotoxicology 5:228–235. doi:10.3109/17435390.2010.502980

Burello E, Worth AP (2015) A rule for designing safer nanomaterials: do not interfere with the cellular redox equilibrium. Nanotoxicology 9(Suppl 1):116–117. doi:10.3109/17435390.2013.828109

Cardillo D, Tehei M, Hossain MS, Islam MM, Bogusz K, Shi D, Mitchell D, Lerch M, Rosenfeld A, Corde S (2016) Synthesis-dependent surface defects and morphology of hematite nanoparticles and their effect on cytotoxicity *in vitro*. ACS Appl Mater Interfaces 8:5867–5876. doi:10.1021/acsami.5b12065

CFS, Center for food safety (2010) Nanotechnology and food safety. Food and Environmental Hygiene Department, HKSAR. http://www.cfs.gov.hk/english/programme/programme_rafs/files/programme_rafs_ft_01_04_Nanotechnology_e.pdf. Accessed 30th Sep 2016

Chae Y, An YJ (2016) Toxicity and transfer of polyvinylpyrrolidone-coated silver nanowires in an aquatic food chain consisting of algae, water fleas, and zebrafish. Aquat Toxicol 173:94–104. doi:10.1016/j.aquatox.2016.01.011

Chen W, Duan L, Zhu DQ (2007) Adsorption of polar and nonpolar organic chemicals to carbon nanotubes. Environ Sci Technol 41:8295–8300. doi:10.1021/es071230h

Chen CL, Hu J, Shao DD, Li JX, Wang XK (2009) Adsorption behavior of multiwall carbon nanotube/iron oxide magnetic composites for Ni (II) and Sr (II). J Hazard Mater 164:923–928. doi:10.1016/j.jhazmat.2008.08.089

Cheng JP, Flahaut E, Cheng SH (2007) Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos. Environ Toxico Chem 26:708–716. doi:10.1897/06-272R.1

Cote I et al (2016) The next generation of risk assessment multi-year study: highlights of findings, applications to risk assessment, and future directions. Environ Health Perspect 124:1671–1682. doi:10.1289/EHP233

Darlington TK, Neigh AM, Spencer MT, Nguyen OT, Oldenburg SJ (2009) Nanoparticle characteristics affecting environmental fate and transport through soil. Environ Toxicol Chem 28:1191–1199. doi:10.1897/08-341.1

De Matteis V, Cascione M, Brunetti V, Toma CC, Rinaldi R (2016) Toxicity assessment of anatase and rutile titanium dioxide nanoparticles: the role of degradation in different pH conditions and light exposure. Toxicol in Vitro 37:201–210. doi:10.1016/j.tiv.2016.09.010

Defra, Department for Environment, Food & Rural Affairs (2009) Voluntary Reporting Scheme for Engineered Nanoscale Materials. http://webarchive.nationalarchives.gov.uk/20130701152729/http://archive.defra.gov.uk/environment/quality/nanotech/policy.htm. Accessed 10th May 2017

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR (1991) Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ Toxicol Chem 10:1541–1583. doi:10.1002/etc.5620101203

ECHA, European Chemicals Agency (2016) Guidance on registration: Version 3.0. https://echa.europa.eu/documents/10162/23036412/registration_en.pdf/de54853d-e19e-4528-9b34-8680944372f2. Accessed 11th May 2017

EEA, European Environment Agency (2001) Late lessons from early warnings: The precautionary principle 1896–2000. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.418.1171&rep=rep1&type=pdf. Accessed 10th May 2017

El-Temsah YS, Joner EJ (2012) Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environ Toxicol 27:42–49. doi:10.1002/tox.20610

EPA, Environmental Protection Agency (2014) Toxic Substances Control Act Section 723.50. https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/low-volume-exemption-new-chemical. Accessed 11th May 2017

Fan WH, Shi ZW, Yang XP, Cui MM, Wang XL, Zhang DF, Liu H, Guo L (2012) Bioaccumulation and biomarker responses of cubic and octahedral Cu_2O micro/nanocrystals in *Daphnia magna*. Water Res 46:5981–5988. doi:10.1016/j.watres.2012.08.019

Farré M, Sanchís J, Barceló D (2011) Analysis and assessment of the occurrence, the fate and the behavior of nanomaterials in the environment. TrAC Trends Analyt Chem 30:517–527. doi:10.1016/j.trac.2010.11.014

Forest V, Leclerc L, Hochepied JF, Trouvé A, Sarry G, Pourchez J (2017) Impact of cerium oxide nanoparticles shape on their *in vitro* cellular toxicity. Toxicol in Vitro 38:136–141. doi:10.1016/j.tiv.2016.09.022

Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS (2007) Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility. Environ Sci Technol 41:8484–8490. doi:10.1021/es071445r

Geller W, Müller H (1981) The filtration apparatus of Cladocera: filter mesh-sizes and their implications on food selectivity. Oecologia 49:316–321. doi:10.1007/BF00347591

Gophen M, Geller W (1984) Filter mesh size and food particle uptake by *Daphnia*. Oecologia 64:408–412. doi:10.1007/BF00379140

Gottschalk F, Nowack B (2011) The release of engineered nanomaterials to the environment. J Environ Monit 13:1145–1155. doi:10.1039/C0EM00547A

Gottschalk F, Sonderer T, Scholz RW, Nowack B (2009) Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ Sci Technol 43:9216–9222. doi:10.1021/es9015553

Gottschalk F, Ort C, Scholz RW, Nowack B (2011) Engineered nanomaterials in rivers-exposure scenarios for Switzerland at high spatial and temporal resolution. Environ Pollut 159:3439–3445. doi:10.1016/j.envpol.2011.08.023

Gottschalk F, Sun TY, Nowack B (2013) Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. Environ Pollut 181:287–300. doi:10.1016/j.envpol.2013.06.003

Grieger KD, Baun A, Owen R (2010) Redefining risk research priorities for nanomaterials. J Nanopart Res 12:383–392. doi:10.1007/s11051-009-9829-1

Gruene P, Rayner DM, Redlich B, van der Meer AF, Lyon JT, Meijer G, Fielicke A (2008) Structures of neutral Au_7, Au_{19}, and Au_{20} clusters in the gas phase. Science 321:674–676. doi:10.1126/science.1161166

Hadioui M, Merdzan V, Wilkinson KJ (2015) Detection and characterization of ZnO nanoparticles in surface and waste waters using single particle ICPMS. Environ Sci Technol 49:6141–6148. doi:10.1021/acs.est.5b00681

Handy RD, von der Kammer F, Lead JR, Hassellöv M, Owen R, Crane M (2008) The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology 17:287–314. doi:10.1007/s10646-008-0199-8

Hansen SF, Baun A (2012) European regulation affecting nanomaterials - review of limitations and future recommendations. Dose-Response 10:364–383. doi:10.2203/dose-response.10-029.Hansen

Hansen SF, Maynard A, Baun A, Tickner JA (2008a) Late lessons from early warnings for nanotechnology. Nat Nanotechnol 3:444–447. doi:10.1038/nnano.2008.198

Hansen SF, Michelson ES, Kamper A, Borling P, Stuer-Lauridsen F, Baun A (2008b) Categorization framework to aid exposure assessment of nanomaterials in consumer products. Ecotoxicology 17:438–447. doi:10.1007/s10646-008-0210-4

Hansen SF, Larsen BH, Olsen SI, Baun A (2009) Categorization framework to aid hazard identification of nanomaterials. Nanotoxicology 1:243–250. doi:10.1080/17435390701727509

Harrison RM, Harrad S, Lead J (2003) Global disposition of contaminants. In: Hoffman DJ, Rattner BA, Burton J, Allen G, Cairns J, John (eds) Handbook of ecotoxicology, 2nd edn. Lewis Publishers, Boca Raton, FL, pp 855–875

Hartmann G, Schuster M (2013) Species selective preconcentration and quantification of gold nanoparticles using cloud point extraction and electrothermal atomic absorption spectrometry. Anal Chim Acta 761:27–33. doi:10.1016/j.aca.2012.11.050

Hartmann NB, Jensen KA, Baun A, Rasmussen K, Rauscher H, Tantra R, Cupi D, Gilliland D, Pianella F, Riego Sintes JM (2015) Techniques and protocols for dispersing nanoparticle powders in aqueous media: is there a rationale for harmonization? J Toxicol Environ Health B 18:299–326. doi:10.1080/10937404.2015.1074969

He XJ, Sanders S, Aker WG, Lin YF, Douglas J, H-m H (2016) Assessing the effects of surface-bound humic acid on the phototoxicity of anatase and rutile TiO_2 nanoparticles *in vitro*. J Environ Sci 42:50–60. doi:10.1016/j.jes.2015.05.028

Heinlaan M, Ivask A, Blinova I, Dubourguier H-C, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71:1308–1316. doi:10.1016/j.chemosphere.2007.11.047

Hendren CO, Mesnard X, Droge J, Wiesner MR (2011) Estimating production data for five engineered nanomaterials as a basis for exposure assessment. Environ Sci Technol 45:2562–2569. doi:10.1021/es103300g

Hjorth R, Hansen SF, Jacobs M, Tickner J, Ellenbecker M, Baun A (2017a) The applicability of chemical alternatives assessment for engineered nanomaterials. Integr Environ Assess Manag 13:177–187. doi:10.1002/ieam.1762

Hjorth R, Holden PA, Hansen SF, Colman BP, Grieger K, Hendren CO (2017b) The role of alternative testing strategies in environmental risk assessment of engineered nanomaterials. Environ Sci: Nano 4: 292–301. doi:10.1039/c6en00443a

Hoecke KV, Quik JTK, Mankiewicz-Boczek J, Schamphelaere KAC, Karel AC, Elsaesser A, Meeren PV, Barnes C, McKerr G, Howard CV, Meent DV (2009) Fate and effects of CeO_2 nanoparticles in aquatic ecotoxicity tests. Environ Sci Technol 43:4537–4546. doi: 10.1021/es9002444

Holden PA et al (2016) Considerations of environmentally relevant test conditions for improved evaluation of ecological hazards of engineered nanomaterials. Environ Sci Technol 50:6124–6145. doi:10.1021/acs.est.6b00608

Hotze EM, Phenrat T, Lowry GV (2010) Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. J Environ Qual 39:1909–1924. doi:10.2134/jeq2009.0462

Hristozov DR, Gottardo S, Critto A, Marcomini A (2012) Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology 6:880–898. doi:10.3109/17435390.2011.626534

Hsu A, Liu FZ, Leung YH, Ma APY, Djurišić AB, Leung FCC, Chan WK, Lee HK (2014) Is the effect of surface modifying molecules on antibacterial activity universal for a given material? Nano 6:10323–10331. doi:10.1039/C4NR02366H

Hund-Rinke K, Baun A, Cupi D, Fernandes TF, Handy R, Kinross JH, Navas JM, Peijnenburg W, Schlich K, Shaw BJ, Scott-Fordsmand JJ (2016) Regulatory ecotoxicity testing of nanomaterials - proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles. Nanotoxicology 10:1442–1447. doi:10.1080/17435390.2016.1229517

ISO, International Organization for Standardization (2015) Nanotechnologies-Vocabulary-Part2:Nano-objects. http://www.iso.org/iso/home/store/catalogue_ics/catalogue_detail_ics.htm?csnumber=54440. Accessed 30th Sep 2016

Iswarya V, Bhuvaneshwari M, Alex SA, Iyer S, Chaudhuri G, Chandrasekaran PT, Bhalerao GM, Chakravarty S, Raichur AM, Chandrasekaran N (2015) Combined toxicity of two crystalline phases (anatase and rutile) of titania nanoparticles towards freshwater microalgae: *Chlorella sp*. Aqua Toxicol 161:154–169. doi:10.1016/j.aquatox.2015.02.006

Iswarya V, Bhuvaneshwari M, Chandrasekaran N, Mukherjee A (2016) Individual and binary toxicity of anatase and rutile nanoparticles towards *Ceriodaphnia dubia*. Aqua Toxicol 178:209–221. doi:10.1016/j.aquatox.2016.08.007

Jarošová B, Filip J, Hilscherová K, Tuček J, Šimek Z, Giesy JP, Zbořil R, Bláha L (2015) Can zero-valent iron nanoparticles remove water-borne estrogens? J Environ Manag 150:387–392. doi:10.1016/j.jenvman.2014.12.007

Ji J, Long Z, Lin D (2011) Toxicity of oxide nanoparticles to the green algae *Chlorella sp*. Chem Eng J 170:525–530. doi:10.1016/j.cej.2010.11.026

Johnson AC, Bowes MJ, Crossley A, Jarvie HP, Jurkschat K, Jurgens MD, Lawlor AJ, Park B, Rowland P, Spurgeon D, Svendsen C, Thompson IP, Barnes RJ, Williams RJ, Xu N (2011) An assessment of the fate, behaviour and environmental risk associated with sunscreen TiO_2 nanoparticles in UK field scenarios. Sci Total Environ 409:2503–2510. doi:10.1016/j.scitotenv.2011.03.040

Juberg DR et al (2017) FutureTox III: bridges for translation. Toxicol Sci 155:22–31. doi:10.1093/toxsci/kfw194

Karlsson HL, Gustafsson J, Cronholm P, Möller L (2009) Size-dependent toxicity of metal oxide particles—a comparison between nano-and micrometer size. Toxicol Lett 188:112–118. doi:10.1016/j.toxlet.2009.03.014

Keller AA, Wang HT, Zhou DX, Lenihan HS, Cherr G, Cardinale BJ, Miller R, Ji ZX (2010) Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environ Sci Technol 44: 1962–1967. doi:10.1021/es902987d

Kennedy AJ, Hull MS, Steevens JA, Dontsova KM, Chappell MA, Gunter JC, Weiss CA (2008) Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. Environ Toxicol Chem 27:1932–1941. doi:10.1897/07-624.1

Klain SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR (2008) Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem 27:1825–1851. doi:10.1897/08-090.1

Knudsen TB et al (2015) FutureTox II: *in vitro* data and in silico models for predictive toxicology. Toxicol Sci 143:256–267. doi:10.1093/toxsci/kfu234

Kumar CSSR (2006) Biotoxicity of metal oxide nanoparticles. In: Fond AM, Meyer GJ (eds) Nanomaterials – toxicity, health and environmental issues. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany

Kumar N, Shah V, Walker VK (2012) Influence of a nanoparticle mixture on an arctic soil community. Environ Toxicol Chem 31:131–135. doi:10.1002/etc.721

Kvitek L, Panáček A, Soukupova J, Kolář M, Večeřová R, Prucek R, Holecova M, Zbořil R (2008) Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). J Phys Chem C 112:5825–5834. doi:10.1021/jp711616v

Kwak JI, An YJ (2016) Trophic transfer of silver nanoparticles from earthworms disrupts the locomotion of springtails (Collembola). J Hazard Mater 315:110–116. doi:10.1016/j.jhazmat.2016.05.005

Kwok KWH, Leung KMY, Flahaut E, Cheng JP, Cheng SH (2010) Chronic toxicity of double-walled carbon nanotubes to three marine organisms: influence of different dispersion methods. Nanomedicine 5:951–961. doi:10.2217/nnm.10.59

Leung YH, Chan CM, Ng AM, Chan HT, Chiang MW, Djurišić AB, Ng YH, Jim WY, Guo MY, Leung FCC, Chan WK, Au DT (2012) Antibacterial activity of ZnO nanoparticles with a modified surface under ambient illumination. Nanotechnology 23:475703. doi:10.1088/0957-4484/23/47/475703

Leung YH et al (2015) Toxicity of CeO_2 nanoparticles - the effect of nanoparticle properties. J. Photochem Photobiol B Biol 145:48–59

Li MH, Huang CP (2011) The responses of *Ceriodaphnia dubia* toward multi-walled carbon nanotubes: effect of physical–chemical treatment. Carbon 49:1672–1679. doi:10.1016/j.carbon.2010.12.052

Li Y, Zhang W, Niu J, Chen Y (2012) Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles. ACS Nano 6:5164–5173. doi:10.1021/nn300934k

Limbach LK, Bereiter R, Müller E, Krebs R, Gälli R, Stark WJ (2008) Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency. Environ Sci Technol 42:5828–5833. doi:10.1021/es800091f

Linkov I, Satterstrom FK (2008) Nanomaterial risk assessment and risk management. In: Linkov I, Ferguson E, Magar VS (eds) Real-time and deliberative decision making. NATO Science for peace and security series C: environmental security. Springer, Dordrecht. doi: 10.1007/978-1-4020-9026-4_8

Linkov I, Satterstrom FK, Monica JC Jr, Hansen SF, Davis TA (2009) Nano risk governance: current developments and future perspectives. Nanotech L & Bus 6:203–220

Liu JF, Chao JB, Liu R, Tan ZQ, Yin YG, Wu Y, Jiang GB (2009) Cloud point extraction as an advantageous preconcentration approach for analysis of trace silver nanoparticles in environmental waters. Anal Chem 81:6496–6502. doi:10.1021/ac900918e

Liu N, Li K, Li X, Chang Y, Feng YL, Sun XJ, Cheng Y, Wu ZJ, Zhang HY (2016) Crystallographic facet-induced toxicological responses by faceted titanium dioxide nanocrystals. ACS Nano 10:6062–6073. doi:10.1021/acsnano.6b01657

Majedi SM, Lee HK, Kelly BC (2012) Chemometric analytical approach for the cloud point extraction and inductively coupled plasma mass spectrometric determination of zinc oxide nanoparticles in water samples. Anal Chem 84:6546–6552. doi:10.1021/ac300833t

Malloy TF (2011) Nanotechnology regulation: a study in claims making. ACS Nano 5:5–12. doi:10.1021/nn103480e

Maynard AD (2011) Don't define nanomaterials. Nature 475:31–31. doi:10.1038/475031a

Metcalfee C, Bennettm E, Chappell M, Steevens J, Depledge M, Goss G, Goudey S, Kaczmar S, O'Brien N, Picado A (2009) Strategic management and assessment of risks and toxicity of engineered Nanomaterials (SMARTEN). In: Linkov I, Steevens J (eds) Nanomaterials: risks and benefits, 1st edn. Springer, Dordrecht, pp 95–109

Mitrano DM, Barber A, Bednar A, Westerhoff P, Higgins CP, Ranville JF (2012a) Silver nanoparticle characterization using single particle ICP-MS (SP-ICP-MS) and asymmetrical flow field flow fractionation ICP-MS (AF4-ICP-MS). J Anal At Spectrom 27:1131–1142. doi:10.1039/C2JA30021D

Mitrano DM, Lesher EK, Bednar A, Monserud J, Higgins CP, Ranville JF (2012b) Detecting nanoparticulate silver using single-particle inductively coupled plasma-mass spectrometry. Environ Toxicol Chem 31:115–121. doi:10.1002/etc.719

Mordor Intelligence (2016) Global nanomaterials market-segmented by product type, end-user industry, and geography-trends and forecasts (2015–2020). http://www.researchandmarkets.com/research/qltbs2/global. Accessed 30 Sep 2016

Morose G (2010) The 5 principles of "Design for Safer Nanotechnology". J Clean Prod 18:285–289. doi:10.1016/j.jclepro.2009.10.001

Mu Y, Wu F, Zhao Q, Ji R, Qie T, Zhou Y, Hu Y, Pang C, Hristozov D, Giesy JP, Xing B (2016) Predicting toxic potencies of metal oxide nanoparticles by means of nano-QSARs. Nanotoxicology 10:1207–1214. doi:10.1080/17435390.2016.1202352

Mueller NC, Nowack B (2008) Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol 42:4447–4453. doi:10.1021/es7029637

Mwangi JN, Wang N, Ingersoll CG, Hardesty DK, Brunson EL, Li H, Deng B (2012) Toxicity of carbon nanotubes to freshwater aquatic invertebrates. Environ Toxicol Chem 31:1823–1830. doi:10.1002/etc.1888

Neal C, Jarvie H, Rowland P, Lawler A, Sleep D, Scholefield P (2011) Titanium in UK rural, agricultural and urban/industrial rivers: geogenic and anthropogenic colloidal/sub-colloidal sources and the significance of within-river retention. Sci Total Environ 409:1843–1853. doi:10.1016/j.scitotenv.2010.12.021

Neal AL, Kabengi N, Grider A, Bertsch PM (2012) Can the soil bacterium *Cupriavidus necator* sense ZnO nanomaterials and aqueous Zn^{2+} differentially? Nanotoxicology 6:371–380. doi:10.3109/17435390.2011.579633

Nel A, Xia T, Mädler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311:622–627. doi:10.1126/science.1114397

Nel A, Xia T, Meng H, Wang X, Lin SJ, Ji ZX, Zhang HY (2013) Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and high-throughput screening. Acc Chem Res 46:607–621. doi:10.1021/ar300022h

Nowack B, Bucheli TD (2007) Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut 150:5–22. doi:10.1016/j.envpol.2007.06.006

Oberdörster G, Stone V, Donaldson K (2007) Toxicology of nanoparticles: a historical perspective. Nanotoxicology 1:2–25. doi:10.1080/17435390701314761

OECD, Organisation for Economic Co-operation and Development (2009) Preliminary review of OECD test guidelines for their applicability to manufactured nanomaterials http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2009)21. Accessed 10th May 2017

OECD, Organisation for Economic Co-operation and Development (2010) List of manufactured nanomaterials and list of endpoints for phase one of the sponsorship programme for the testing of manufactured nanomaterials: revision. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2010)46&doclanguage=en. Accessed 12 Dec 2017

OECD, Organisation for Economic Co-operation and Development (2012) Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. http://www.oecd.org/env/ehs/nanosafety/publicationsintheseriesonthesafetyofmanufacturednanomaterials.html. Accessed 3rd Feb 2017

OECD, Organisation for Economic Co-operation and Development (2014) Ecotoxicology and environmental fate of manufactured nanomaterials: Test guidelines. http://www.oecd.org/env/ehs/nanosafety/publicationsintheseriesonthesafetyofmanufacturednanomaterials.html.

OECD, Organisation for Economic Co-operation and Development (2015) Landfilling of Waste Containing Nanomaterials and Nanowaste. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/EPOC/WPRPW(2014)5/FINAL&docLanguage=En. Accessed 12th Oct 2016

Park MVDZ, Neigh AM, Vermeulen JP, de la Fonteyne LJJ, Verharen HW, Briedé JJ, van Loveren H, de Jong WH (2011) The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 32:9810–9817. doi:10.1016/j.biomaterials.2011.08.085

Piccinno F, Gottschalk F, Seeger S, Nowack B (2012) Industrial production quantities and uses of ten engineered nanomaterials in europe and world. J Nanopart Res 14:1109–1120. doi:10.1007/s11051-012-1109-9

Rowlands JC, Sander M, Bus JS, Committee FTO (2014) FutureTox: building the road for 21st century toxicology and risk assessment practices. Toxicol Sci 137:269–277. doi:10.1093/toxsci/kft252

SCENIHR, Scientific Committee on Emerging and Newly Identified Health Risks (2007) The appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_010.pdf. Accessed 11th May 2017

SCENIHR, Scientific Committee on Emerging and Newly Identified Health Risks (2009) Risk assessment of products of nanotechnologies. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_023.pdf. Accessed 11th May 2017

Schmid K, Riediker M (2008) Use of nanoparticles in Swiss industry: a targeted survey. Environ Sci Technol 42:2253–2260. doi:10.1021/es071818o

Simon-Deckers A, Loo S, Mayne-L'hermite M, Herlin-Boime N, Menguy N, Reynaud C, Gouget B, Carrière M (2009) Size-, composition-and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. Environ Sci Technol 43:8423–8429. doi:10.1021/es9016975

Snyder EM, Snyder SA, Giesy JP, Blonde SA, Hurlburt GK, Summer CL, Mitchell RR, Bush DM (2000) SCRAM: a scoring and ranking system for persistent, bioaccumulative, and toxic substances for the north American Great Lakes. Environ Sci Pollut Res 7:52–61. doi:10.1007/BF03028072

Sprung M, Rose U (1988) Influence of food size and food quantity on the feeding of the mussel *Dreissena polymorpha*. Oecologia 77:526–532. doi:10.1007/BF00377269

Su GY, Zhang XW, Giesy JP, Musarrat J, Saquib Q, Alkhedhairy AA, Yu HX (2015) Comparison on the molecular response profiles between nano zinc oxide (ZnO) particles and free zinc ion using a genome-wide toxicogenomics approach. Environ Sci Pollut Res 22:17434–17442. doi:10.1007/s11356-015-4507-6

Sun H, Zhang XZ, Niu Q, Chen YS, CJ C (2006) Enhanced accumulation of arsenate in carp in the presence of titanium dioxide nanoparticle. Water Air Soil Pollut 178:245–254. doi: 10.1007/s11270-006-9194-y

Sun TY, Gottschalk F, Hungerbuhler K, Nowack B (2014) Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. Environ Pollut 185:69–76. doi:10.1016/j.envpol.2013.10.004

Taghon GL (1982) Optimal foraging by deposit-feeding invertebrates: roles of particle size and organic coating. Oecologia 52:295–304. doi:10.1007/BF00367951

Tang Z, Zhao XL, Zhao TH, Wang H, Wang PF, Wu FC, Giesy JP (2016) Magnetic nanoparticles interaction with humic acid: in the presence of surfactants. Environ Sci Technol 50:8640–8648. doi:10.1021/acs.est.6b01749

Tong Z, Bischoff M, Nies L, Applegate B, Turco RF (2007) Impact of fullerene (C60) on a soil microbial community. Environ Sci Technol 41:2985–2991. doi:10.1021/es061953l

Tong Z, Bischoff M, Nies LF, Myer P, Applegate B, Turco RF (2012) Response of soil microorganisms to as-produced and functionalized single-wall carbon nanotubes (SWNTs). Environ Sci Technol 46:13471–13479. doi:10.1021/es303251r

Tourinho PS, van Gestel CA, Lofts S, Svendsen C, Soares AM, Loureiro S (2012) Metal-based nanoparticles in soil: fate, behavior, and effects on soil invertebrates. Environ Toxicol Chem 31:1679–1692. doi:10.1002/etc.1880

Tuoriniemi J, Cornelis G, Hassellov M (2012) Size discrimination and detection capabilities of single-particle ICPMS for environmental analysis of silver nanoparticles. Anal Chem 84:3965–3972. doi:10.1021/ac203005r

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF Jr, Rejeski D, Hull MS (2015) Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory. Beilstein J Nanotechnol 6:1769–1780. doi:10.3762/bjnano.6.181

von der Kammer F, Legros S, Hofmann T, Larsen EH, Loeschner K (2011) Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation. TrAC Trends Analyt Chem 30:425–436. doi:10.1016/j.trac.2010.11.012

von der Kammer F, Ferguson PL, Holden PA, Masion A, Rogers KR, Klaine SJ, Koelmans AA, Horne N, Unrine JM (2012) Analysis of engineered nanomaterials in complex matrices (environment and biota): general considerations and conceptual case studies. Environ Toxicol Chem 31:32–49. doi:10.1002/etc.723

Warheit DB (2008) How meaningful are the results of Nanotoxicity studies in the absence of adequate material characterization? Toxicol Sci 101:183–185. doi:10.1093/toxsci/kfm279

Weinberg AM (1985) Science and its limits: the regulator's dilemma. Issues Sci Technol 2:59–72. doi: http://www.jstor.org/stable/43310360

Westerhoff P, Song G, Hristovski K, Kiser MA (2011) Occurrence and removal of titanium at full scale wastewater treatment plants: implications for TiO_2 nanomaterials. J Environ Monitor 13:1195–1203. doi:10.1039/C1EM10017C

Wong SWY, Leung KMY (2014) Temperature-dependent toxicities of nano zinc oxide to marine diatom, amphipod and fish in relation to its aggregation size and ion dissolution. Nanotoxicology 8(Suppl 1): 24–35. doi:10.3109/17435390.2013.848949

Wong SWY, Leung PTY, Djurišić AB, Leung KMY (2010) Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. Anal Bioanal Chem 396:609–618. doi:10.1007/s00216-009-3249-z

Wong SWY, Leung KMY, Djurišić AB (2013) A comprehensive review on the aquatic toxicity of engineered nanomaterials. Rev Nanosci Nanotechnol 2:79–105. doi:10.1166/rnn.2013.1025

Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nel AE (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2:2121–2134. doi:10.1021/nn800511k

Xiong D, Fang T, Yu L, Sima X, Zhu W (2011) Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. Sci Total Environ 409:1444–1452. doi:10.1016/j.scitotenv.2011.01.015

Xiu ZM, Zhang QM, Puppala HL, Colvin VL, Alvarez PJ (2012) Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett 12:4271–4275. doi:10.1021/nl301934w

Yang K, Xing BS (2007) Desorption of polycyclic aromatic hydrocarbons from carbon nanomaterials in water. Environ Pollut 145:529–537. doi:10.1016/j.envpol.2006.04.020

Yin LY, Cheng YW, Espinasse B, Colman BP, Auffan M, Wiesner M, Rose J, Liu J (2011) More than the ions: the effects of silver nanoparticles on *Lolium multiflorum*. Environ Sci Technol 45:2360–2367. doi:10.1021/es103995x

Yung MMN et al (2015a) Salinity-dependent toxicities of zinc oxide nanoparticles to the marine diatom *Thalassiosira pseudonana*. Aquat Toxicol 165:31–40. doi:10.1016/j.aquatox.2015.05.015

Yung MMN, Mouneyrac C, Leung KMY (2015b) Ecotoxicity of zinc oxide nanoparticles in the marine environment. Encycl Nanotechnol:2075–2084. doi:10.1007/978-94-007-6178-0_100970-1

Yung MMN et al (2017) Influences of temperature and salinity on physicochemical properties and toxicity of zinc oxide nanoparticles to the marine diatom *Thalassiosira pseudonana*. Sci Rep. doi:10.1038/s41598-017-03889-1

Zhang HY et al (2012) Use of metal oxide nanoparticle band gap to develop a predictive paradigm for acute pulmonary inflammation based on oxidative stress. ACS Nano 6:4349–4368. doi:10.1021/nn3010087

Zhao XL, Liu SL, Wang PF, Tang Z, Niu HY, Cai YQ, Wu FC, Wang H, Meng W, Giesy JP (2015) Surfactant-modified flowerlike layered double hydroxide-coated magnetic nanoparticles for preconcentration of phthalate esters from environmental water samples. J Chromatogr A 1414:22–30. doi:10.1016/j.chroma.2015.07.105

Zhu X, Wang J, Zhang X, Chang Y, Chen Y (2010) Trophic transfer of TiO_2 nanoparticles from *Daphnia* to zebrafish in a simplified freshwater food chain. Chemosphere 79:928–933. doi:10.1016/j.chemosphere.2010.03.022

Supplementary Information

Regulation of Engineered Nanomaterials: Current Challenges, Insights and Future Directions

Racliffe W. S. Lai[1], Katie W. Y. Yeung[1], Mana M. N. Yung[1], Aleksandra B. Djurišić[2], John P. Giesy[1,3,4], Kenneth M. Y. Leung[1,5]*

1. The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China
2. Department of Physics, The University of Hong Kong, Pokfulam, Hong Kong, China
3. Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
4. State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China
5. State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

* Corresponding author: Prof. Kenneth M. Y. Leung

Corresponding Address:	School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China
Corresponding Tel. No.:	+852 2299 0607
Corresponding Fax No.:	+852 2517 6082
Corresponding Email:	kmyleung@hku.hk

1. Current regulations on ENMs

1.1 Australia

Cosmetics

Almost all cosmetic ingredients, including natural ingredients such as oils, are regulated as industrial chemicals under the Industrial Chemicals (Notification and Assessment) Act 1989 (NICNAS 2016). Nanomaterials are defined as materials that are intentionally produced, manufactured or engineered materials with unique properties and size range between 1 nm and 100 nm (NICNAS 2016). Labelling of cosmetics ingredients need to follow strict standards. However, it does not mention the case of nanomaterials.

Drugs

Although drugs are regulated under the Therapeutic Goods Act 1989 which states that drugs should be sold safely with quality, there are still no specific regulations or standards on nanomaterials used in drugs. (Federal Register of Legislation 2005).

Food

Australia New Zealand Food Standards Code (ANZF Code), which is operated and regulated under Food Standards Australia New Zealand (FSANZ), sets out requirements for food labelling and selling of food, food packaging and labelling in Australia. However, nanotechnology and nanomaterials used in food or food packaging are not mentioned in FSANZ. Only new substances added in food require pre-market approval which means if nanomaterials are added in food, it must undergo safety assessment before appearing in the market (Ludlow 2009).

Pesticides

Australian Pesticide and Veterinary Medicines Agency (APVMA) is responsible to regulate pesticide and veterinary medicine chemicals in Australia. APVMA published a report “Nanotechnologies for pesticides and veterinary medicines: Regulatory considerations” in 2015 that includes definitions of nanomaterials used in pesticides and veterinary medicines (APVMA 2015b). However, it does not include detailed guidelines on registration and regulation of products that include nanomaterials because APVMA does not dare to announce and provide guideline before having more information on the risk and toxicity of nanomaterials in pesticides (APVMA 2015b). In case there are products containing nanomaterials, applicants should contact APVMA for guidance first before registration (APVMA 2015a). Risk assessment framework is being considered to be modified in order to address the pesticides which may contain engineered nanomaterials.

1.2 Canada

According to Policy Statement on Health Canada’s Working Definition for Nanomaterial, nanomaterials refer to any manufactured products or materials at or within 1 – 100 nm, inclusive, in at least one external dimension, or has internal or surface structure at this size range (Health Canada 2011).

Cosmetics

Cosmetics sold in Canada must meet the requirements of Food and Drugs Act and Cosmetics Regulations C.R.C., c. 869 under Health Canada. All ingredients presence in cosmetics must be labelled clearly, but the Cosmetics Regulations does not mention anything related to nanomaterials used in cosmetics such as toxicity, labelling and risk assessment (Health Canada 2007).

Drugs and Food

Health Canada regulated food and drugs under Food and Drugs Act and Food and Drug Regulations (C. R. C., c. 870), indicating that no one in the Canadian market can sell food that contains harmful substances and unfit to human health, and sell drugs that are manufactured under unsanitary conditions (Health Canada 2016b). All ingredients presence in drugs and food must be labelled clearly, however, nanomaterials used in food and drugs are not included in the Act and there is no regulation and statement concerning the labelling of nanomaterials on drugs and food or even food packaging.

Pesticides

The Pest Management Regulatory Agency (PMRA) of Health Canada is responsible to protect human health and safety to the environment while using pesticides. Pesticides used in Canada are controlled by Pest Control Products Regulations (SOR /2006-124) under Pest Control Products Act (Health Canada 2016a). The application of pesticides in Canada is strictly controlled. Toxicological data and exposure assessment have to be reported and submitted during registration (Health Canada 2016a). Guidance on toxicology evaluation and occupational exposure is provided with clear indication of ingredients used in pesticides, but it is still lack of information about nanomaterials used in pesticides.

1.3 Europe (including UK)

Europe (including UK) is the leading place to monitor and regulate the use of chemicals among the communities. Due to increasing use of nanotechnology in recent decades, the Commission of the European Communities adopted an incremental approach to regulate the use of nanotechnology on consumer products, including cosmetics, drugs, food and pesticides (Hansen & Baun 2012). Chemicals in Europe (including UK) are simply regulated under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) which was effective from June 1, 2007 (Brazell 2012). However, the application and registration of nanomaterials on products are not specifically mentioned under REACH (Hansen & Baun 2012). There are other regulations and guidance for monitoring nanomaterials.

Cosmetics

Directive 76/768/EEC is the old Cosmetics Directive implemented in 1976 with a list of cosmetic products in Annex I that are approved to be used in the market while there is a list of prohibited substances used in cosmetics in Annex II, although it does not include the size of particles used and restrict the use of nanomaterials in cosmetics (Commission of European Communities 2008). With the various definitions of nanomaterials between the European Parliament and the Council of the European Union, the new Regulation (EC) No 1223/2009 completely replaces Directive 76/768/EEC from July 11, 2013 with the internationally agreed definition of nanomaterials in cosmetics regulation that nanomaterials refer to insoluble or bio-persistent, and intentionally manufactured materials with one or more external dimensions with 1 to 100 nm (EP and CEU 2009b, art. 2). This new regulation requires manufacturers to inform the European Commission about the presence of nanomaterials in the form of physical property, chemical property, quantity, toxicological profile and reasonably foreseeable exposure conditions in electronic means six months prior their sale in the market (EP and CEU 2009b, art. 16). For cosmetics that are already in the market, the presence of nanomaterials in the products has to be labelled separately in the list of ingredients on the packaging with "nano" in brackets (e.g. ZnO [nano], silver [nano]) (EP and CEU 2009b, art. 19, 40; Hansen & Baun 2012). Since there is still a lack of information on the toxicity of nanomaterials, Scientific Committee on Consumer Safety (SCCS) has to provide guidance in testing methodologies to characterize the nanomaterials and provide opinion on the safety issue of nanomaterials used in cosmetics when appropriate (EP and CEU 2009b, part 30).

Drugs

Reflection paper on nanotechnology-based medicinal products for Human Use published by European Medicines Agency defines nanotechnology as "the production and application of structures devices and systems by controlling the shape and size of materials at nano-meter scale at atomic level of around 0.2 nm (2 Å) to around 100 nm" while nano-medicine refers to the application of nanotechnology in making medicines (European Medicines Agency 2006). Although a marketing authorization application is required for nano-medicines before circulating in the market, the use of nanomaterials in medicine is not regulated in the Directive 2001/83/EC due to the lack of data on effects of chronic exposure on health of humans and environmental fate of nano-medicines (European Medicines Agency 2006; EP and CEU 2012). Directive 2001/83/EC stated that the characteristics and ingredients of medicines have to be clearly stated on the package of medicines (EP and CEU 2012).

Food

Regulation (EC) No 178/2002 is one of the food and food packaging regulations in Europe; however, none of them regulate the application of nanomaterials in food products (EP and CEU 2002). Although materials used in food have to be stated on packaging, there is still no law requiring the label of "nano" on the ingredient list to indicate the presence of nanomaterials in food (EP and CEU 2002; FOE 2008). Moreover, it is difficult to do hazard assessment on food with nanomaterials because there are still no validated methods and no standard definition of nanomaterials for the detection of nanomaterials in food (EFSA 2011; FOE 2008). FOE (2008) suggested that the size of nanomaterials should be extended to 300 nm from 100 nm to have a better health and environment assessment. With publication of "Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain" by EFSA, definition of nanomaterials, requirements of nanomaterials used, test methods on the toxicity of products as well as the approval list of nanomaterials to be used in the market have been clearly stated (EFSA 2011). In this guidance, nanomaterial refers to a material with at least one size measurement between approximately 1 and 100 nm (EFSA 2011). This guidance only serves as a guideline for consumers or manufacturers on the use of nanomaterials in food but it is not a law such that the government does not have enforcement power to monitor the use of nanomaterials in food industry.

Pesticides

Regulation EC 1107/2009 is based on the precautionary principle, in order to make sure that active products in pesticides will not adversely affect human and animal health as well as the environment (EP and CEU 2009a, art. 1). It requires active ingredients that in pesticides to be assessed at community level, to be undergone registration and follow legal procedure. Although this regulation does not include any reference and guidance on nanotechnology applied in pesticides, "Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain" developed a practical approach which was used in assessing the potential risks of nanoscience and nanotechnologies (EFSA 2011). According to the guidance, nanomaterials refer to a material with at least one size measurement between approximately 1 nm and 100 nm (EFSA 2011). It provides guideline on physicochemical characteristics of engineered nanomaterials used in food, feed additives, and pesticides; it also provides guidance on testing methods to identify, characterize and quantify nanomaterials (EFSA 2011). Risk assessment should be performed if there are uncertainties. Directive 98/8/EC is another regulation regulating the use of biocides, and non-agricultural pesticides, in community because they are active substances which contain one or more active substances that prevent the action on any harmful organism by chemical or biological means and have potential to harm human, animal health and the environment (EP and CEU 1998, art. 2). This regulation does not include any nano-related risk and hazards assessment as well as the necessarily of labelling.

1.4 Japan

Cosmetics

Cosmetics are regulated under Pharmaceutical Affairs Law (The Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices as the amended title) by the Ministry of Health, Labour and Welfare (MHLW) (MHLW 2013). Marketing license for cosmetics is needed for all cosmetics to be sold in the market. All ingredients used in cosmetics have to be labelled on the packaging. There are lists of positive and negative ingredients with the usable one stated in the former list while prohibiting ingredients included in the latter list (MHLW 2013). However, nanomaterials used in cosmetics are not stated in the law but they must be approved by MHLW for the use in cosmetics.

Drugs and Food

Drugs and food are originally regulated under the Pharmaceutical Affairs Law, but it changed to The Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices starting from November 2014 (MHLW 2013). Marketing license for drugs is needed in order to be sold in the market, but not for food. However, no matter in either old or new law, there are still no particular regulations on the use of nanomaterials in food and drugs. If there are cases using nanomaterials, MHLW will handle it on case-by-case basis instead of regulating it by law (MHLW 2013).

Pesticides

Pesticides are mainly applied in agriculture in Japan. Therefore, the application of pesticides is monitored under Ministry of Health, Labour and Welfare (MHLW

2013). It mainly focuses on the maximum residue limits for agricultural chemicals in food and it does not include any information about the use of nanomaterials in pesticides (USDA Foreign Agricultural Service 2009).

1.5 The United States

There are several federal agencies in the United States to monitor and manage toxic and harmful materials, and products circulating in the market, including Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), the Occupational Safety and Health Administration (OSHA) and the Consumer Product Safety Commission (CPSC) (Beaudrie et al. 2013). Generally, chemical substances are regulated under Toxic Substances Control Act (TSCA) while pesticides are regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Food, drugs and cosmetics are regulated under the Federal Food, Drug, and Cosmetic Act (FFDCA). These three Acts will go through pre-market assessment and management such that chemical substances, pesticides, food and drugs are evaluated before entering the market (Beaudrie et al. 2013). Existing chemical substances in the market have to be maintained safe while new chemical substances have to undergo risk assessment before circulating in the market. Cosmetics will go through post-market assessment such that manufacturers have the responsibility to review and ensure the safety of products circulating in the market (Beaudrie et al. 2013).

Cosmetics

In the United States, cosmetics are regulated under Federal Food, Drug, and Cosmetic Act. In 2014, Food and Drug Administration (FDA) issued a “Guidance for Industry – Safety of Nanomaterials in Cosmetic Products” which provides guidelines when nanomaterials have to be used in cosmetics (FDA 2014). The guidance generally classifies nanomaterial as a material that is engineered to have at least one external dimension, or an internal or surface structure, in the nanoscale range with approximately 1 nm to 100 nm (FDA 2014). Manufacturers are highly suggested to contact FDA to undergo safety assessment before production. Also, the guideline does not require the use of nanomaterials in cosmetics to undergo pre-market review (FDA 2014). Manufacturers must follow strict regulations for cosmetics labelling, but it does not mention anything about the labelling of nanomaterials if they are presence in the product.

Drugs

Drugs and public health in the United States are regulated under Public Health Service Act (FDA 2009). However, it does not include any definitions, risk health assessment or monitoring on the use of nanomaterials in pharmaceutical industry.

Food

Although food is regulated under Federal Food, Drug and Cosmetics Act in the United States by FDA, it does not include any definitions, risk health assessment or monitoring on the use of nanomaterials in food products. Instead, FDA formed Nanotechnology Task Force in August 2006 in order to encourage the continuous development and application of this innovative nanotechnology in products by regulation and monitoring (FDA 2015a). It also reviews and evaluates the possible adverse effect of nanomaterials to human health. A "Guidance for Industry on the Use of Nanomaterials in Food for Animals" was issued in August 2015 (FDA 2015b). It stated that nanomaterial is a material that is engineered to have at least one external dimension, or an internal or surface structure, in the nanoscale range with approximately 1 nm to 100 nm (FDA 2015b). This Guidance does not include any regulatory status but instead it serves as a guideline and recommendation for the industry on the use of nanomaterials in animal food (FDA 2015b). All food additives require pre-market approval based on data demonstrating safety. However, if food additives are determined to be “generally recognized as safe” by the manufacturers, they can be exempted from the pre-market approval (Brazell 2012). Ingredients presence in food additives have to be labelled clearly to ensure the safety.

Pesticides

In the United States, the registration, distribution and use of pesticides are governed by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). All pesticides used or sold in the United States have to be registered with EPA. However, it does not mention any regulation on nanomaterials in pesticides.

2. Prioritizing ENMs for regulations

Figure S1 summarized the worldwide production of nanomaterials and the ranking was based on median of the summarized production volumes (SiO_2 > TiO_2 > FeO_x > ZnO > carbon nanotube > Ag > CeO_2 > fullerene > quantum dots) **(Table S1a-i)**. Due to the paucity of studies found, literatures from a large time span of a decade between 2007 and 2016 were adopted and multiple locations instead of purely global statistics. To minimize data variability due to such wide selection, production volumes representing only a specific country or economic region were extrapolated to worldwide production in proportion to respective Gross Domestic Product (GDP) (Sun et al. 2014). Same approach was used to further extrapolate the worldwide production from the year of the paper published to 2015, the latest year with available GDP statistics. However, huge variability in terms of several magnitudes was still obtained. This variability may be due to the different methodology adopted by different researchers as has been discussed in the paper.

The ranking of application of ENMs in different commercial products (Ag > TiO_2 > SiO_2 > Si > C > carbon nanotube > ZnO > Organics > Au > ceramics) is shown in **Figure S2** and **Table S2.** It represents the prevalence of ENMs and hence an implication for the likelihood and ubiquitousness of ENMs in daily life and environment. It serves as an implementation to above ranking of production volume in evaluation of ENM exposure. It was obtained from the largest online databank (http://www.nanotechproject.org/) currently available, enlisting 1,814 commercial products from 32 countries that claimed to be ENM-incorporated. However, the inventory only summarized a small portion of the products while the majority of the nanomaterial-incorporated products do not state the ENMs they are applying.

Further, toxicities of ENMs that occurred in above two rankings were summarized from different studies **(Figure S3, Table S3a-d)**. The median lethal concentration (LC_{50}) and median effective concentration (EC_{50}) of ENMs were recorded. The review focused on four models commonly used in different toxicity evaluation are selected: *Pseudokirchneriella subcapitata*, *Daphnia magna*, *Danio rerio* and mammalian cells (different cell lines are pooled together as a reflection of nano-toxicity towards mammals). To formulate valid comparison, only toxicity values from same test conditions are adopted: 72h acute toxicity test for *P. subcapitata*, 48h acute toxicity test for *D. magna*, 96h acute toxicity test for *D. rerio* and 24h acute toxicity for mammalian cells. But not all the ENMs from above rankings have been studied for their toxicity. Some ENMs that were not specified clearly were not surveyed, including carbons, organics and ceramics. They were classified into different hazard categories based on the median of their LC_{50} and EC_{50} values (Bondarenko et al. 2013). *Daphnia magna* was found to be most sensitive to the ENMs surveyed: Ag > Fe_2O_3 > ZnO > TiO_2 > CeO_2 while mammalian cells were generally more tolerant to the ENMs and it followed the sequence as Ag > CeO_2 > ZnO > TiO_2. The sensitivity of *P. subcapitata* followed as Ag > ZnO > CeO_2, > SiO_2 > TiO_2 while the one of *D. rerio* followed the sequence of Ag > ZnO > TiO_2. Ag was found to be the most toxic ENM towards all four model organisms among the ENMs in the ranking.

ENMs were selected into the prioritized list if it occurs in the ranking of either the production volume or the prevalence in the market and is ranked as 'toxic', 'very toxic' or 'extremely toxic' in the toxicity ranking. The criteria are to meet the exposure and toxicity components in the conventional risk assessment framework. From this point of view, ENMs that require immediate and further investigation for future regulations are: nanoparticles of Ag, ZnO, Fe_2O_3 and TiO_2.

Relative research effort has been compared with reference to the number of research articles available on the search engine Web of Science **(Figure S4, Table S4)**. The search was conducted by a key word search using the name of each of the common ENMs. Apparently, more research efforts were made for carbon nanotubes and Ag nanoparticles, and followed by TiO_2 (**Figure S4**).

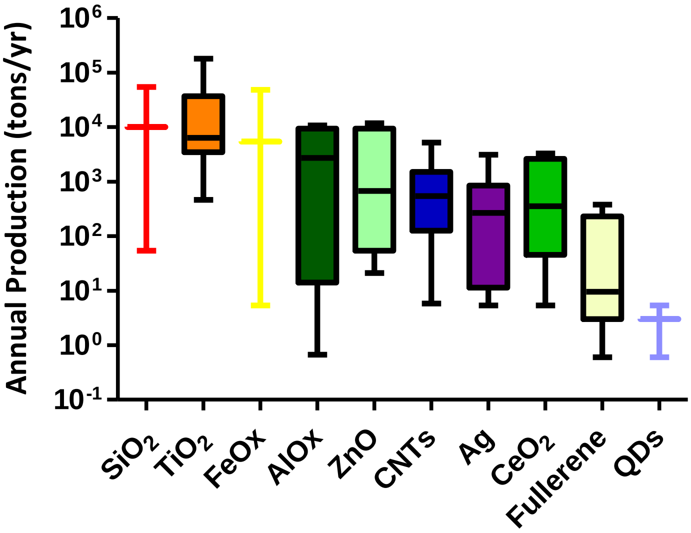

Figure S1 Ranking of annual production of different ENMs.

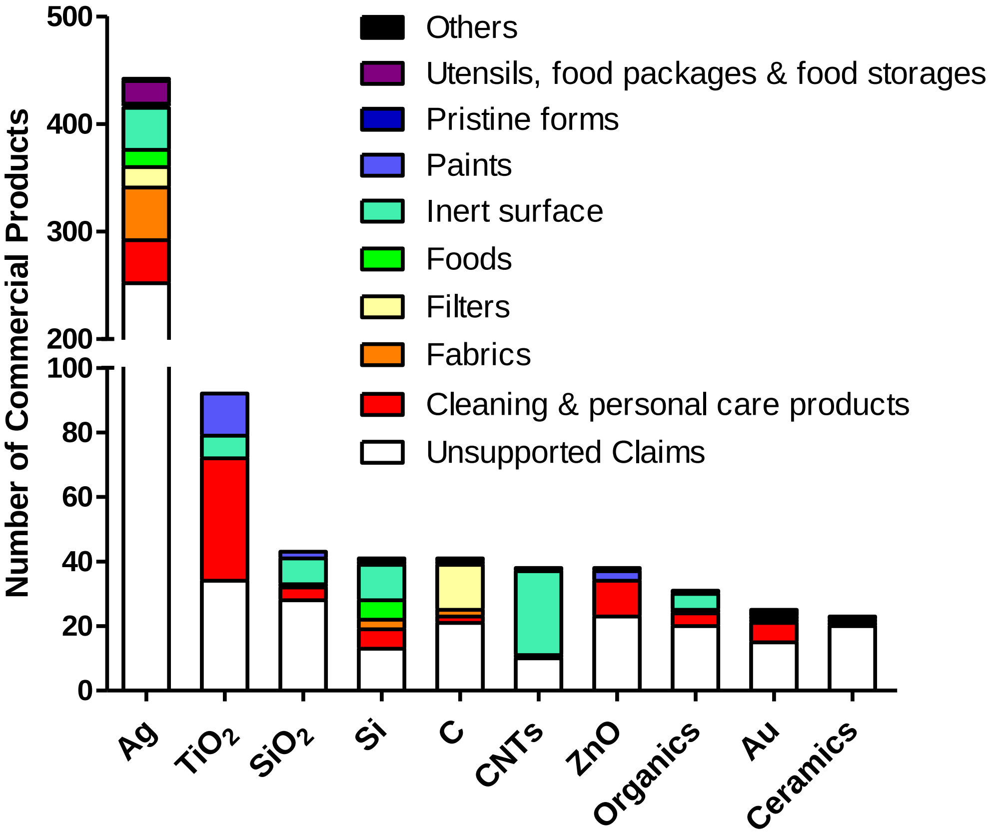

Figure S2 Ranking of usage of ENMs in different commercial products.

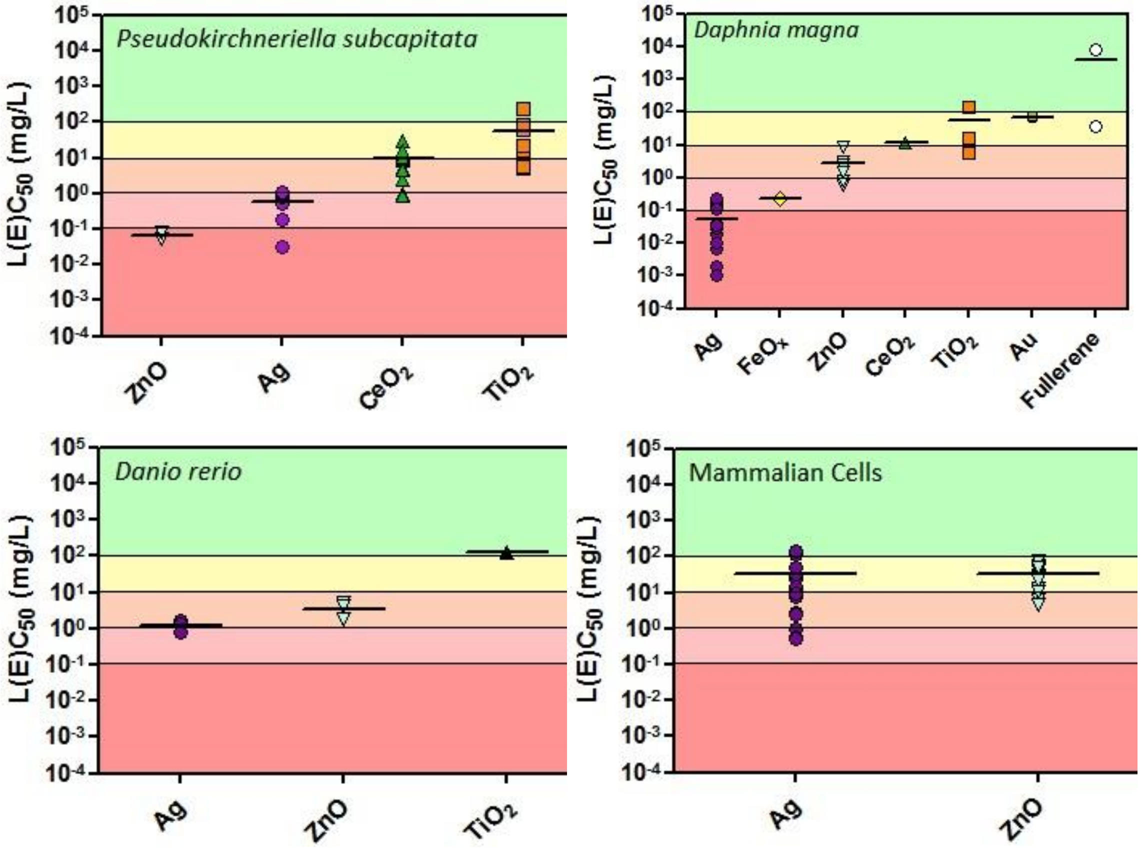

Figure S3 Ranking of potential toxicities of different ENMs. Different colour groups imply the hazard categories for the ENMs based on their median LC_{50} and EC_{50} values, ranging from 'extremely toxic' (< 0.1 mg/L), 'very toxic' (0.1-1 mg/L), 'toxic' (1-10 mg/L), 'harmful' (10-100 mg/L) and 'not classified' (> 100mg/L), modified from the study of Bondarenko et al. (2013).

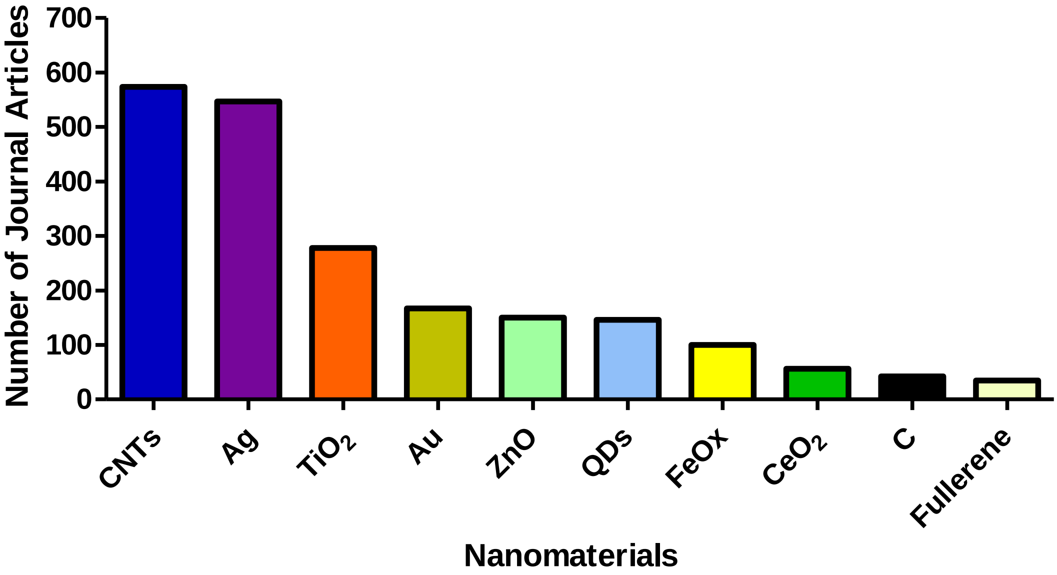

Figure S4 Ranking of research effort input for different ENMs.

Table S1 Production volume of different ENMs

(a) SiO_2

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2008	Switzerland	75.4	10,053.3	Schmid and Riediker (2008)
2012	World	55 – 55,000	54.4 – 54,355.9	Piccinno et al. (2012)

(b) TiO_2

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2009	United States	44,400	22,633.8	Robichaud et al. (2009)
2011	United States	7,800 – 38,000	36,945.5 – 179,991.1	Hendren et al. (2011)
2008	Switzerland	435.5	58,032.7	Schmid and Riediker (2008)
2014	European Union	10,000	39,695.1	Sun et al. (2014)
2014	China	1,850	13,136.7	Estimation from chart in Gao et al. (2013)
2012	World	550 – 5,500	543.6 – 5,435.6	Piccinno et al. (2012)
2008	World	400 – 5,000	466.0 – 5,825.4	Mueller and Nowack (2008)
2007	World	679	867.3	Sourcing from references in Gottschalk et al. (2009)
2008	World	3,000	3,495.2	
2007	World	5,000	6,386.8	
2008	World	5,000	5,825.4	
2010	World	12,500	14,003.2	USEPA (2010)

(c) FeO_x

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2008	Switzerland	365	48,642.0	Schmid and Riediker (2008)
2012	World	5.5 – 5,500	5.4 – 5,435.6	Piccinno et al. (2012)

(d) AlO_x

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2008	Switzerland	0.005	0.667	Schmid and Riediker (2008)
2014	China	1,510	10,722.4	Estimation from chart in Gao et al. (2013)
2012	World	55 – 5,500	54.4 – 5,435.6	Piccinno et al. (2012)

(e) ZnO

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2008	Switzerland	70	9,377.2	Schmid and Riediker (2008)
2014	European Union	1,600	6,351.2	Sun et al. (2014)
2014	China	1,660	11,787.5	Estimation from chart in Gao et al. (2013)
2012	World	55 – 550	54.4 – 543.6	Piccinno et al. (2012)
2007	World	528	674.4	Sourcing from references in Gottschalk et al. (2009)
2008	World	1,800	2,097.1	
2008	World	18	21.0	

2007	World	20	25.5	
2010	World	528	591.5	Zhang and Saebfar (2010)
2011	World	10,000	10,094.0	Aschberger et al. (2011)

(f) Carbon Nanotubes				
Year of Publication	**Region Covered**	**Estimated Production (tonnes)**	**Extrapolated Worldwide Production based on 2015 GDP (tonnes)**	**Reference**
2011	United States	55 – 1,101	260.5 – 5,215.0	Hendren et al. (2011)
2008	Switzerland	1	133.3	Schmid and Riediker (2008)
2014	European Union	400	1,587.8	Sun et al. (2014)
2012	World	55 – 550	54.4 – 543.6	Piccinno et al. (2012)
2008	World	350 – 500	407.8 – 582.5	Mueller and Nowack (2008)
2011	World	1,000	1,009.4	Farré et al. (2011)
2008	World	295	343.7	Köhler et al. (2008)
2008	World	426	496.3	Sourcing from references in Gottschalk et al. (2009)
2007	World	278	355.1	

(g) Ag				
Year of Publication	**Region Covered**	**Estimated Production (tonnes)**	**Extrapolated Worldwide Production based on 2015 GDP (tonnes)**	**Reference**
2011	United States	2.8 – 20	13.3 – 94.7	Hendren et al. (2011)
2012	World	5.5 – 550	5.4 – 543.6	Piccinno et al. (2012)
2008	Switzerland	3.1	413.1	Schmid and Riediker (2008)
2008	World	500 – 1,230	582.5 – 1,433.0	Mueller and Nowack (2008)
2008	World	5	5.8	Sourcing from references in

2008	World	563	655.9	Gottschalk et al. (2009)
2014	European Union	30	119.1	Sun et al. (2014)
2014	China	440	3,124.4	Estimation from chart in Gao et al. (2013)

(h) CeO_2

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2011	United States	35 – 700	165.8 – 3,315.6	Hendren et al. (2011)
2012	World	5.5 – 550	5.4 – 543.6	Piccinno et al. (2012)

(i) Fullerene

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2011	United States	2 – 80	9.5 – 378.9	Hendren et al. (2011)
2014	European Union	20	79.4	Sun et al. (2014)
2012	World	0.6 – 5.5	0.6 – 5.4	Piccinno et al. (2012)

(j) Quantum Dots

Year of Publication	Region Covered	Estimated Production (tonnes)	Extrapolated Worldwide Production based on 2015 GDP (tonnes)	Reference
2008	Switzerland	0.6 – 5.5	0.6 – 5.4	Schmid and Riediker (2008)

Table S2 Inventory of commercial products containing nanomaterials. Source: http://www.nanotechproject.org/cpi/

Product Category	**Number of Products Containing Engineered Nanomaterials**									
	Ag	**TiO_2**	**SiO_2**	**Si**	**C**	**Carbon nanotubes**	**ZnO**	**Organics**	**Au**	**Ceramics**
Unsupported Claim	252	34	28	13	21	10	23	20	15	20
Fabrics	49		1	3	2	1		1		1
Foods	16			6					1	
Utensils, food packages and food storages	21			1			1			
Inert surface	39	7	8	11		26		5		1
Cleaning and personal care products	40	38	4	6	2		11	4	6	
Filters	19				14					
Others	2							1	2	
Paints	1	13	2		1	1	3			1
Pristine forms	3			1	1				1	
Sum	442	92	43	41	41	38	38	31	25	23

Table S3 Toxicity data of different nanomaterials towards the four models

(a) *Daphnia magna*

Nanomaterials	**Endpoint**	**Experimental Time**	**L(E)C$_{50}$ (mg/L)**	**Particle Size**	**Remarks**	**References**
Ag	EC50	48 h	0.001	60		Kim et al. (2011)
Ag	EC50	48 h	0.03	<100	Coated with lactate	Zhao and Wang (2012)
Ag	EC50	48 h	0.02	8.4±2.8	Coated with polyvinylpyrrolidone	Blinova et al. (2013)
Ag	EC50	48 h	0.04	12.5±4	Coated with protein	Blinova et al. (2013)
Ag	LC50	48 h	0.003-0.004			Li et al. (2010)
Ag	LC50	48 h	0.007	10		Li et al. (2010)
Ag	LC50	48 h	0.01	20		Li et al. (2010)
Ag	LC50	48 h	0.02	30		Li et al. (2010)
Ag	LC50	48 h	0.03	50		Li et al. (2010)
Ag	LC50	48 h	0.01	10		Ivask et al. (2014)
Ag	LC50	48 h	0.034	20		Ivask et al. (2014)
Ag	LC50	48 h	0.141	40		Ivask et al. (2014)
Ag	LC50	48 h	0.168	60		Ivask et al. (2014)
Ag	LC50	48 h	0.218	80		Ivask et al. (2014)
Ag	LC50	48 h	0.115	<15	Coated with polyvinylpyrrolidone	Tuominen et al. (2013)
Ag	LC50	48 h	0.002	10-20	Coated with polyvinylpyrrolidone	Zhao and Wang (2012)
Ag	LC50	48 h	0.001	10-20	Coated with sodium dodecylbenzene sulfonate	Zhao and Wang (2012)
Au	LC50	48 h	65-75			Li et al. (2010)
CeO$_2$	LC50	48 h	12			García et al. (2011)
Fe$_3$O$_4$	LC50	48 h	0.23			García et al. (2011)
Fullerene (C$_{60}$)	LC50	48 h	7900		Dispersed by sonication	Lovern and Klaper (2006)
Fullerene (C$_{60}$)	LC50	48 h	> 35			Zhu et al. (2006)
TiO$_2$	LC50	48 h	16			García et al. (2011)

TiO_2	LC50	48 h	5.5			(Lovern and Klaper 2006)
TiO_2	LC50	48 h	143.387	<20	> 99.5% anatase	Zhu et al. (2009)
ZnO	EC50	48 h	0.6	20		Zhu et al. (2009)
ZnO	EC50	48 h	3.2	50-70		Blinova et al. (2010)
ZnO	EC50	48 h	2.6	<100		Blinova et al. (2010)
ZnO	EC50	48 h	0.8	40		Hai-zhou et al. (2012)
ZnO	EC50	48 h	2.1	50-70		Naddafi et al. (2011)
ZnO	LC50	48 h	8.8	50-70		Heinlaan et al. (2008)
ZnO	LC50	48 h	1.5	20		Zhu et al. (2009)
ZnO	LC50	48 h	1.5	40		Hai-zhou et al. (2012)

(b) *Pseudokirchneriella subcapitata*

Nanomaterials	**Endpoint**	**Experimental Time**	**L(E)C_{50} (mg/L)**	**Particle Size**	**Remarks**	**References**
Ag	IC50	72 h	0.18	10		Ivask et al. (2014)
Ag	IC50	72 h	0.52	20		Ivask et al. (2014)
Ag	IC50	72 h	0.82	40		Ivask et al. (2014)
Ag	IC50	72 h	0.94	60		Ivask et al. (2014)
Ag	IC50	72 h	1.14	80		Ivask et al. (2014)
Ag	IC50	72 h	0.0324	3-8		Ribeiro et al. (2014)
CeO_2	EC50	72 h	12.8	12		Rodea-Palomares et al. (2011)
CeO_2	EC50	72 h	0.95	13		Rodea-Palomares et al. (2011)
CeO_2	EC50	72 h	0.88	22		Rodea-Palomares et al. (2011)
CeO_2	EC50	72 h	8.96	28		Rodea-Palomares et al. (2011)

CeO_2	EC50	72 h	12.3	12		Rodea-Palomares et al. (2011)
CeO_2	EC50	72 h	5.2	13		Rodea-Palomares et al. (2011)
CeO_2	EC50	72 h	2.4	22		Rodea-Palomares et al. (2011)
CeO_2	EC50	72 h	8.5	28		Rodea-Palomares et al. (2011)
CeO_2	IC50	72 h	29.6	12		Rodea-Palomares et al. (2011)
CeO_2	IC50	72 h	9.7	13		Rodea-Palomares et al. (2011)
CeO_2	IC50	72 h	4.4	22		Rodea-Palomares et al. (2011)
CeO_2	IC50	72 h	10.3			Rodea-Palomares et al. (2011)
CeO_2	IC50	72 h	16.4	28		Rodea-Palomares et al. (2011)
TiO_2	EC50	72 h	5			Hall et al. (2009)
TiO_2	EC50	72 h	9.72			Aruoja et al. (2009)
TiO_2	IC50	72 h	241	10		Hartmann et al. (2010)
TiO_2	IC50	72 h	71.1	30		Hartmann et al. (2010)
TiO_2	IC50	72 h	16		Coated with aluminium	Menard et al. (2011)
TiO_2	IC50	72 h	61			Menard et al. (2011)
TiO_2	IC50	72 h	21			Menard et al. (2011)
TiO_2	IC50	72 h	87			Menard et al. (2011)
TiO_2	IC50	72 h	5.83	25-70		Menard et al. (2011)
ZnO	EC50	72 h	0.052	50-70		Aruoja et al. (2009)
ZnO	IC50	72 h	0.085	30		Franklin et al. (2007)

(c) ***Danio rerio***

Nanomaterials	**Endpoint**	**Experimental Time**	**L(E)C_{50} (mg/L)**	**Particle Size**	**Remarks**	**References**

Ag	EC50	96 h	1.61	35		Wang et al. (2012)
Ag	EC50	96 h	1.36	80	Coated with polyvinylpyrrolidone	Wang et al. (2012)
Ag	EC50	96 h	0.78	15		Wang et al. (2012)
TiO_2	LC50	96 h	124.5	20-70		Xiong et al. (2011)
ZnO	LC50	96 h	4.92	30		Xiong et al. (2011)
ZnO	LC50	96 h	3.97	30		Yu et al. (2011)
ZnO	LC50	96 h	1.79	20		Yu et al. (2011)

(d) Mammalian Cells

Nanomaterials	**Cell Type**	**Endpoint**	**Experimental Time**	**L(E)C_{50} (mg/L)**	**Particle Size**	**Remarks**	**References**
Ag	A549 cells	EC50	24 h	1	5	Coated with polyvinylpyrrolidone	Foldbjerg et al. (2011)
Ag	A549 cells	EC50	24 h	10	20	Coated with polyvinylpyrrolidone	Foldbjerg et al. (2011)
Ag	A549 cells	EC50	24 h	13.3	50	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	A549 cells	IC50	24 h	11.25	30-50	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	A549 cells	IC50	24 h	7.5	30-50	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	HepG2 cells	EC50	24 h	0.59	5	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	HepG2 cells	EC50	24 h	25.5	20	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	HepG2 cells	EC50	24 h	33.6	50	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	HepG2 cells	IC50	24 h	2.9	5-10		Liu et al. (2010)
Ag	MCF-7 cells	EC50	24 h	0.5	5	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	MCF-7 cells	EC50	24 h	14.3	20	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	MCF-7 cells	EC50	24 h	47.6	50	Coated with	Liu et al. (2010)

						polyvinylpyrrolidone	
Ag	SGC-7901 cells	EC50	24 h	9.9	5	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	SGC-7901 cells	EC50	24 h	50.9	20	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	SGC-7901 cells	EC50	24 h	112	50	Coated with polyvinylpyrrolidone	Liu et al. (2010)
Ag	THP-1 cells	EC50	24 h	2.44	69±3	Coated with polyvinylpyrrolidone	Foldbjerg et al. (2009)
Ag	THP-1 cells- derived macrophages	IC50	24 h	110	20	Coated with peptide	Haase et al. (2012)
Ag	THP-1 cells- derived macrophages	IC50	24 h	140	40	Coated with peptide	Haase et al. (2012)
ZnO	A549 cells	IC50	24 h	52	<50		Seiffert et al. (2011)
ZnO	A549 cells	IC50	24 h	54	<50		Seiffert et al. (2011)
ZnO	A549 cells	IC50	24 h	45	<50		Seiffert et al. (2011)
ZnO	A549 cells	IC50	24 h	56	<50		Seiffert et al. (2011)
ZnO	Ana-1 cells	IC50	24 h	40.3	70.89		Song et al. (2010)
ZnO	Ana-1 cells	IC50	24 h	30.9	19		Song et al. (2010)
ZnO	BEAS-2B cells	IC50	24 h	6.5	22.6±5.1		Zhang et al. (2012a)
ZnO	BEAS-2B cells	IC50	24 h	4.5	22.6±5.1		Zhang et al. (2012a)
ZnO	CCL-131 cells	IC50	24 h	75	50-70		Jeng and Swanson (2006)
ZnO	CCL-131 cells	IC50	24 h	13.5	50-70		Jeng and Swanson (2006)

ZnO	cultured rat alveolar epithelial cell monolayers	IC50	24 h	13.7	20		Kim et al. (2010)
ZnO	cultured rat alveolar epithelial cell monolayers	IC50	24 h	22.5	20		Kim et al. (2010)
ZnO	MH-S cells	IC50	24 h	45.4	71		Zhang et al. (2012b)
ZnO	MH-S cells	IC50	24 h	26.7	19		Zhang et al. (2012b)
ZnO	MH-S cells	IC50	24 h	13.6	70±13		Zhang et al. (2012b)
ZnO	RAW 264.7 cells	IC50	24 h	21.7	22.6+5.1		Zhang et al. (2012a)
ZnO	RAW 264.7 cells	IC50	24 h	10.1	22.6+5.1		Zhang et al. (2012a)
ZnO	skin fibroblasts	IC50	24 h	49	50-70		Dechsakulthorn et al. (2007)

Table S4 Number of research articles about nanomaterials available in toxicological field. Source: Web of Science

Nanomaterials	**Number of Articles in Toxicological Studies**
Carbon nanotube	574
Ag	547
TiO_2	278
Au	167
ZnO	150
Quantum Dots	146
FeO_x	100
CeO_2	56
C	42
Fullerene	35
SiO_2	12
AlO_x	8
Si	4

Cited References

APVMA (Australian Pesticide and Veterinary Medicines Agency). (2015a). Products of nanotechnology. http://apvma.gov.au/node/97. Accessed on August 3, 2016.

APVMA (Australian Pesticide and Veterinary Medicines Agency). (2015b). Nanotechnologies for pesticides and veterinary medicines: Regulatory considerations.

Aruoja V, Dubourguier HC, Kasemets K, Kahru A (2009) Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae Pseudokirchneriella subcapitata. Sci Total Environ 407:1461-1468. doi:10.1016/j.scitotenv.2008.10.053

Aschberger K, Micheletti C, Sokull-Klüttgen B, Christensen FM (2011) Analysis of currently available data for characterising the risk of engineered nanomaterials to the environment and human health — Lessons learned from four case studies. Environ Int 37:1143-1156

Beaudrie, C. E. H., Kandlikar, M., & Satterfield, T. (2013). From cradle-to-grave at the nanoscale: Gaps in U.S. regulatory oversight along the nanomaterial life cycle. Environ Sci & Technol, 47(11), 5524-5534. doi:10.1021/es303591x

Blinova I et al. (2013) Toxicity of two types of silver nanoparticles to aquatic crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Environ Sci Pollut R 20:3456-3463

Blinova I, Ivask A, Heinlaan M, Mortimer M, Kahru A (2010) Ecotoxicity of nanoparticles of CuO and ZnO in natural water. Environl Pollut 158:41-47

Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A (2013) Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: A critical review. Arch Toxicol 87:1181-1200. doi:10.1007/s00204-013-1079-4

Brazell L. (2012). Nanotechnology Law. Wolters Kluwer.

Commission of European Communities. (2008). Council directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC).

Dechsakulthorn F, Hayes A, Bakand S, Joeng L, Winder C (2007) In vitro cytotoxicity assessment of selected nanoparticles using human skin fibroblasts. AATEX 14:397-400

EFSA (European Food Safety Authority). (2011). Endorsed for public consultation draft scientific opinion guidance on risk assessment concerning potential risks arising from applications of nanoscience and nanotechnologies to food and feed. EFSA Scientific Committee, European Food Safety Authority (EFSA), Parma, Italy

EP and CEU (European Parliament and Council of the European Union). (1998). Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market.

EP and CEU (European Parliament and Council of the European Union). (2002). Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L 31-1-L 31/24.

EP and CEU (European Parliament and Council of the European Union). (2009a). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

EP and CEU (European Parliament and Council of the European Union). (2009b). Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (1) Official Journal of the European Union L 342 Volume 52 22 December 200 ISSN 1725-2555 L 342/59 L 342/209

EP and CEU (European Parliament and Council of the European Union). (2012). Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use

European Medicines Agency. (2006). European Medicines Agency: Reflection paper on nanotechnology-based medicinal products for human use (EMEA/CHMP/70769/2006). London: European Medicines Agency

Farré M, Sanchís J, Barceló D (2011) Analysis and assessment of the occurrence, the fate and the behavior of nanomaterials in the environment. TrAC Trend Analyt Chem 30:517-527. doi:10.1016/j.trac.2010.11.014

FDA (Food and Drug Administration). (2009). Public Health Service Act.

FDA. (2014). Guidance for Industry – Safety of Nanomaterials in Cosmetic Products.

FDA. (2015a). Nanotechnology Task Force. http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/ucm2006658.htm. Accessed on August 3, 2016.

FDA. (2015b). Guidance for Industry Use of Nanomaterials in Food for Animals.

Federal Register of Legislation. (2005). Therapeutic Goods Act 1989.

FOE (Friends of the Earth). (2008). Out of the laboratory and on to our plates nanotechnology in Food & Agriculture. Friends of the Earth Australia, Europe & U.S.A.

Foldbjerg R, Dang DA, Autrup H (2011) Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch Toxicol 85:743-750

Foldbjerg R, Olesen P, Hougaard M, Dang DA, Hoffmann HJ, Autrup H (2009) PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. Toxicol Lett 190:156-162

Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS (2007) Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): The importance of particle solubility. Environ Sci & Technol 41:8484-8490

Gao Y, Luo ZX, He NP, Wang MK (2013) Metallic nanoparticle production and consumption in China between 2000 and 2010 and associative aquatic environmental risk assessment. J Nanopart Res 15:1681-1690. doi:10.1007/s11051-013-1681-7

García A et al. (2011) Acute toxicity of cerium oxide, titanium oxide and iron oxide nanoparticles using standardized tests. Desalination 269:136-141. doi:10.1016/j.desal.2010.10.052

Gottschalk F, Sonderer T, Scholz RW, Nowack B (2009) Modeled Environmental Concentrations of Engineered Nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for Different Regions. Environ Sci & Technol 43:9216-9222. doi:10.1021/es9015553

Haase A et al. (2012) A novel type of silver nanoparticles and their advantages in toxicity testing in cell culture systems. Arch Toxicol 86:1089-1098

Hai-zhou Z, Guang-hua L, Jun X, Shao-ge J (2012) Toxicity of nanoscale CuO and ZnO to *Daphnia magna*. Chem Res Chinese Universites 28:209-213

Hall S, Bradley T, Moore JT, Kuykindall T, Minella L (2009) Acute and chronic toxicity of nano-scale TiO_2 particles to freshwater fish, cladocerans, and green algae, and effects of organic and inorganic substrate on TiO_2 toxicity. Nanotoxicology 3:91-97. doi:10.1080/17435390902788078

Hansen, S. F., & Baun, A. (2012). European regulation affecting nanomaterials - Review of limitations and future recommendations. Dose-Response, 10(3), 364-383. doi:10.2203/dose-response.10-029.Hansen

Hartmann N, Von der Kammer F, Hofmann T, Baalousha M, Ottofuelling S, Baun A (2010) Algal testing of titanium dioxide nanoparticles—testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology 269:190-197

Health Canada. (2007). Cosmetics Regulations C.R.C., c.869.

Health Canada. (2011). Policy statement on Health Canada's working definition for nanomaterial. http://www.hc-sc.gc.ca/sr-sr/pubs/nano/pol-eng.php. Accessed on August 1, 2016.

Health Canada. (2016a). Pest Control Products Regulations (SOR /2006-124)

Health Canada. (2016b). Food and Drugs Act C.R.C., c.870.

Heinlaan M, Ivask A, Blinova I, Dubourguier H-C, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO 2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and Thamnocephalus platyurus. Chemosphere 71:1308-1316

Hendren CO, Mesnard X, Droge J, Wiesner MR (2011) Estimating production data for five engineered nanomaterials as a basis for exposure assessment. Environ Sci Technol 45:2562-2569. doi:10.1021/es103300g

Ivask A et al. (2014) Size-dependent toxicity of silver nanoparticles to bacteria, yeast, algae, crustaceans and mammalian cells in vitro. PloS one 9:e102108

Jeng HA, Swanson J (2006) Toxicity of metal oxide nanoparticles in mammalian cells. J Environ Sci Health A 41:2699-2711

Kim J, Kim S, Lee S (2011) Differentiation of the toxicities of silver nanoparticles and silver ions to the Japanese medaka *Oryzias latipes* and the cladoceran *Daphnia magna*. Nanotoxicology 5:208-214

Kim YH et al. (2010) Alveolar epithelial cell injury due to zinc oxide nanoparticle exposure. Am J Respir Crit Care Med 182:1398-1409

Köhler AR, Som C, Helland A, Gottschalk F (2008) Studying the potential release of carbon nanotubes throughout the application life cycle. J Clean Prod 16:927-937

Li T et al. (2010) Comparative toxicity study of Ag, Au, and Ag-Au bimetallic nanoparticles on Daphnia magna. Anal Bioanal Chem 398:689-700. doi:10.1007/s00216-010-3915-1

Liu W et al. (2010) Impact of silver nanoparticles on human cells: effect of particle size. Nanotoxicology 4:319-330

Lovern SB, Klaper R (2006) *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. Environ Toxicol Chem 25:1132-1137.

Ludlow, K. (2009). The Readiness of Australian Food Regulation for the use of Nanotechnology in Food and Food Packaging. Faculty of Law, Monash University, Research Paper No 2009/42.

Menard A, Drobne D, Jemec A (2011) Ecotoxicity of nanosized TiO_2. Review of in vivo data. Environl Pollut 159:677-684. doi:http://dx.doi.org/10.1016/j.envpol.2010.11.027

MHLW (Ministry of Health, Labour and Welfare). (2013). Outline of the Law for Partial Revision of the Pharmaceutical Affairs Law (Act No.84 of 2013).

Mueller NC, Nowack B (2008) Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol 42:4447-4453. doi:10.1021/es7029637

Naddafi K, Zare M, Nazmara S (2011) Investigating potential toxicity of phenanthrene adsorbed to nano-ZnO using Daphnia magna. Toxicol Environ Chem 93:729-737

NICNAS (National Industrial Chemicals Notification and Assessment Scheme). (2016). Nanomaterials and nanotechnology. https://www.nicnas.gov.au/news-and-events/Topics-of-interest/subjects/nanomaterials-nanotechnology. Accessed on January 24, 2017.

Piccinno F, Gottschalk F, Seeger S, Nowack B (2012) Industrial production quantities and uses of ten engineered nanomaterials in europe and world. J Nanopart Res 14:1109-1120.

Ribeiro F et al. (2014) Silver nanoparticles and silver nitrate induce high toxicity to *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. Sci Total Environ 466:232-241

Robichaud CO, UYAR AE, Darby MR, Zucker LG, Wiesner MR (2009) Estimates of upper bounds and trends in nano-TiO_2 production as a basis for exposure assessment. Environ Sci & Technol 43:4227-4233. doi:10.1021/es8032549

Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, García-Calvo E, Santiago J, Rosal R (2011) Physicochemical characterization and ecotoxicological assessment of CeO_2 nanoparticles using two aquatic microorganisms. Toxicol Sci 119:135-145

Schmid K, Riediker M (2008) Use of nanoparticles in Swiss industry: A targeted survey. Environ Sci Technol 42:2253-2260. doi:10.1021/es071818o

Seiffert JM, Baradez M-O, Nischwitz V, Lekishvili T, Goenaga-Infante H, Marshall D (2011) Dynamic monitoring of metal oxide nanoparticle toxicity by label free impedance sensing. Chem Res Toxicol 25:140-152

Song W, Zhang J, Guo J, Zhang J, Ding F, Li L, Sun Z (2010) Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. Toxicol Lett 199:389-397

Sun TY, Gottschalk F, Hungerbuhler K, Nowack B (2014) Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. Environl Pollut 185:69-76. doi:10.1016/j.envpol.2013.10.004

Tuominen M, Schultz E, Sillanpää M (2013) Toxicity and stability of silver nanoparticles to the green alga Pseudokirchneriella subcapitata in boreal freshwater samples and growth media. Nanomaterials Environ 1:48. doi:10.2478/nanome-2013-0004

USDA Foreign Agricultural Service. (2009). Pesticide and food safety regulation.

USEPA (2010) Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen (Final). https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid= 230972&CFID=76787711&CFTOKEN=95461732. Accessed 13th Feb 2017

Wang Z, Chen J, Li X, Shao J, Peijnenburg WJ (2012) Aquatic toxicity of nanosilver colloids to different trophic organisms: contributions of particles and free silver ion. Toxicol Environ Chem 31:2408-2413

Xiong D, Fang T, Yu L, Sima X, Zhu W (2011) Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. Sci Total Environ 409:1444-1452

Yu LP, Fang T, Xiong DW, Zhu WT, Sima XF (2011) Comparative toxicity of nano-ZnO and bulk ZnO suspensions to zebrafish and the effects of sedimentation, OH production and particle dissolution in distilled water. J Environ Monitor 13:1975-1982

Zhang HY et al. (2012a) Use of Metal Oxide Nanoparticle band gap to develop a predictive paradigm for acute pulmonary inflammation based on oxidative stress. ACS Nano 6:4349-4368. doi:10.1021/nn3010087

Zhang J, Song W, Guo J, Zhang J, Sun Z, Ding F, Gao M (2012b) Toxic effect of different ZnO particles on mouse alveolar macrophages. J Hazard Mater 219:148-155

Zhang S, Saebfar H (2010) Chemical information call-in candidate: nano zinc oxide. California Dept of Toxic substances control, September:1-11

Zhao CM, Wang WX (2012) Importance of surface coatings and soluble silver in silver nanoparticles toxicity to *Daphnia magna*. Nanotoxicology 6:361-370

Zhu S, Oberdörster E, Haasch ML (2006) Toxicity of an engineered nanoparticle (fullerene, C 60) in two aquatic species, *Daphnia* and fathead minnow. Mar Environ Res 62:S5-S9

Zhu X, Zhu L, Chen Y, Tian S (2009) Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*. J Nanopart Res 11:67-75