Molecular and Integrative Toxicology

Jamie R. Lead
Shareen H. Doak
Martin J.D. Clift *Editors*

Nanotoxicology in Humans and the Environment

Springer

Molecular and Integrative Toxicology

Molecular and Integrative Toxicology presents state-of-the-art toxicology in a useful context. Volumes emphasize the presentation of cellular and molecular information aimed toward the protection of human or animal health or the sustainability of environmental systems. This book series is committed to maintaining the highest level of integrity in the content published. It has a Conflict of Interest policy in place and complies with international, national and/or institutional standards on research involving Human Participants and/or Animals and Informed Consent. The Book series is a member of the Committee on Publication Ethics (COPE) and subscribes to its principles on how to deal with acts of misconduct thereby committing to investigate allegations of misconduct in order to ensure the integrity of research. This book series may use plagiarism detection software to screen the submissions. If plagiarism is identified, the COPE guidelines on plagiarism will be followed.

Content published in this book series is peer reviewed. It solicits reviews from the senior editors of the volume since they are the experts in the field, and they are already known to the authors. In cases, however, where there are particular concerns, a single-blind review system is used where independent external experts are asked for advice. These reviewers know the names of the authors, but the identities of the reviewers are unknown to the authors. Submitted manuscripts are reviewed by at least two experts (the senior editors of the volume and or the consulting editors) as well as the series editor. The reviews evaluate whether the manuscript is scientifically sound and coherent, whether it meets the guidelines regarding style, content, and level of writing. The volume editors decide on the suitability of the manuscript based on their reviews.

More information about this series at http://www.springer.com/series/8792

Jamie R. Lead • Shareen H. Doak
Martin J. D. Clift
Editors

Nanotoxicology in Humans and the Environment

Springer

Editors
Jamie R. Lead
Center for Environmental Nanoscience and Risk
Department of ENHS
University of South Carolina
Columbia, SC, USA

Shareen H. Doak
Institute of Life Science, Ctr. for NanoHealth
Swansea University Medical School
Swansea, Mid Glamorgan, United Kingdom

Martin J. D. Clift
Institute of Life Science, Ctr. for NanoHealth
Swansea University Medical School
Swansea, United Kingdom

ISSN 2168-4219 ISSN 2168-4235 (electronic)
Molecular and Integrative Toxicology
ISBN 978-3-030-79807-9 ISBN 978-3-030-79808-6 (eBook)
https://doi.org/10.1007/978-3-030-79808-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Overview of Nanotoxicology in Humans and the Environment; Developments, Challenges and Impacts

Stephen J. Evans, Paul M. Vecchiarelli, Martin J. D. Clift, Shareen H. Doak, and Jamie R. Lead

Contents

Abstract This chapter provides an overview of the potential human health and environmental impact of nanomaterials (NMs). These unique materials can be produced naturally, incidentally or manufactured and can have numerous effects on human and ecological health. From the perspective of human health, the ultra-small nature of NMs can cause them to be highly reactive and promote adverse interactions at the organ, tissue, and cellular levels. Ecologically, NMs have the potential to pass into the environment at each point in their life cycle. Within the environment NMs undergo chemical, physical or biological processes that will modify their

Authors "Stephen J. Evans" and "Paul M. Vecchiarelli" are contributed equally as first author
Authors "Stephen J. Evans" and "Paul M. Vecchiarelli" are joint corresponding authors
Authors "Martin J. D. Clift", "Shareen H. Doak" and "Jamie R. Lead" contributed equally as senior author

S. J. Evans (✉) · M. J. D. Clift · S. H. Doak
In Vitro Toxicology Group, Swansea University Medical School, Institute of Life Sciences, Swansea, Wales, UK
e-mail: s.j.evans@swansea.ac.uk

P. M. Vecchiarelli (✉)
Center for Environmental Nanoscience and Risk, Arnold School of Public Health, University of South Carolina, Columbia, SC, USA
e-mail: vecchiar@email.sc.edu

J. R. Lead
Center for Environmental Nanoscience and Risk, Department of ENHS, University of South Carolina, Columbia, SC, USA
e-mail: vecchiar@email.sc.edu

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_1

environmental fate and biological effects. The toxicological issues broadly covered in this chapter are discussed in further detail throughout this book.

Keywords Nanosafety · Human health hazard · Environmental hazard · (Eco) Toxicology · Regulation · Risk · Exposure

Introduction

Nanomaterials (NMs) can be produced naturally, incidentally, or by manufacturing and can have numerous effects on human and ecological health. Naturally-formed NMs include colloidal suspensions, such as humic and fulvic acids, proteins and peptides, and hydrated metal oxides, which are found in aquatic environments (Klaine et al. 2018; Lead et al. 2018; Buffle and van Leeuwen 1992; Buffle and van Leeuwen 1993). Of historical note, early work (Cameron 1915) suggested that clays, soil organic matter, metal oxides, and other minerals are important soil constituents. Modern research indicates that these constituents exhibit unique behaviours at nano-scale (Maurice 2010). Incidental releases of atmospheric NMs can occur through combustion or aerosolization (Klaine et al. 2018).

Over the course of the twentieth and twenty-first centuries, the field of nanotechnology has expanded at an exponential rate and with this expansion there has been a rapid increase in the number of novel engineered NMs being developed (Klaine et al. 2018). The annual projected growth rates for major NMs are shown in Table 1, adapted from Jankovic and Plata (2019). These new materials are having a transformative impact on research and development across numerous sectors including electronics, medicine, aerospace, construction and personal care, and are increasingly being used in nanotechnology-enabled products (Vance et al. 2015; Jankovic and Plata 2019). These new developments have been made possible due to the unique, size dependent physico-chemical properties that NMs exert. Some NMs allow for improved thermal or electrical conductivity, catalytic action, tensile strength, super-paramagnetism, controllable colloidal behaviour, and advanced optical properties. Environmental and human health studies have suggested relationships between these properties of NMs and their environmental fate, transport, and bioavailability, which may present a set of novel risks compared to larger particulate or dissolved counterparts (Lead et al. 2018).

Nanotechnology has been used since ancient times, for example, in the dichroic Lycurgus Cup of fourth century Rome which was made of glass interspersed with gold and silver NMs (Beyda et al. 2020). Photography is another application of NMs, in which daguerreotype photographs in the nineteenth century employed light sensitive silver NMs (Schlather et al. 2019). These artistic uses of nanotechnologies led the way to isolation of NMs, such as fullerenes and carbon nanotubes (CNTs) in 1985, and subsequent discovery of their novel properties (Bayda et al. 2019). Nanocomposites and nanohybrids are emerging classes of NMs, which are created by combining NM and non-NM materials or multiple NM materials, respectively (Lead et al. 2018). NMs may be generated in a powder or suspension form, or incorporated into matrices including polymers, building materials and even food stuffs.

Table 1 Non-exhaustive list of nanomaterials with highest estimated 2019 production volumes, projected growth rates for 2015–2025, and the number of technologies developed of each type based on an evaluation of technological readiness levels (Jankovic and Plata 2019)

Nanomaterial	Production (metric tons)	Projected growth rate (2015–2025)	Number of technologies developed
Aluminium oxide	6400–14,650	6–8%	16
Antimony tin oxide	180–410	7–11%	16
Bismuth oxide	52–108	8–11%	14
Carbon nanotubes	685–3500	5–9%	39
Cellulose	735–4149	21–31%	27
Cerium oxide	1177–2172	6–9%	12
Clays	30,000–68,200	3–6%	9
Cobalt oxide	6.5–11.7	5–9%	9
Dendrimers	0.54–2.97	10–20%	18
Diamonds	21.8–31.4	12–15%	9
Fibres	290–628	12–16%	9
Fullerenes	100–183	12–13%	13
Gold	2.2–4.0	7–12%	7
Graphene	7–310	26–43%	35
Iron oxide	24.5–115	19%	14
Magnesium oxide	37–75	13–16%	16
Manganese oxide	3.7–8.0	10–14%	11
Nickel	5.9–47.8	5–20%	8
Quantum dots	0.5–5.0	58%	17
Silicon dioxide	365,000–2,800,000	9–10%	11
Silver	230–560	6–10%	11
Titanium dioxide	38,500–225,000	4–11%	10
Zinc oxide	8440–47,460	6–8%	5
Zirconium oxide	1.739–42,583	3–4%	12

Direct synthesis of NMs (bottom-up) or high-power milling processes which grind bulk products down into NMs (top-down) are two generic approaches to synthesis, and will have substantial effects on environmental footprint, NM yield, use, production costs, and waste generation (Baraton 2002; Yokoyama and Huang 2005; Klaine et al. 2018; Jankovic and Plata 2019). Surface modification, or coating, is further used to influence NM stability, binding, or chemical functionality (Angel et al. 2013; Lead et al. 2018). Studies indicate that synthesis, suspension and coating methods for NMs can each influence toxicity and environmental behaviour (Klaine et al. 2018).

Characterization and metrology of NMs in the laboratory have advanced significantly since the early 2000s. While NM analysis in complex environments including aquatic, terrestrial, and biological media remains a challenge due to strong binding between natural macromolecules and NMs and low resolution of

conventional imaging techniques, a number of innovative developments have emerged to improve these bottlenecks (Lead et al. 2018). Standardized testing media have helped identify new sources of interactions between NMs and their environment (Geitner et al. 2020). Isotopic labelling of NMs has improved quantitation of NM speciation and concentration (Merrifield et al. 2017; Merrifield et al. 2018) as well as biological uptake (Croteau et al. 2011; Handy et al. 2012; Al-Jubory and Handy 2013; Croteau et al. 2014). Conjugated separation techniques such as inductively coupled plasma, mass spectrometry, and field flow fractionation have improved separation and yielded novel single-cell and single-particle analytical techniques (Merrifield et al. 2017; Merrifield et al. 2018). Physico-chemical characterization has also been standardized for determining how NM properties behave in terms of stability and biological uptake (Liu et al. 2013, 2016).

Potential NM uses are vast and influenced by their physico-chemistry, as shown in Fig. 1, with several nanotechnologies already developed and tested. For example, the size and shape of nano-titanium dioxide (TiO_2) in anatase and rutile crystal structures gives improved photocatalytic properties over bulk TiO_2 (Chaturvedi et al. 2012).

In medicine, a range of NMs including iron oxides and quantum dots are being applied in tissue engineering, imaging enhancement and drug delivery systems (Cortajarena et al. 2014; Jankovic and Plata 2019). Within aerospace and other industries, the light weight and extremely high tensile strength of NMs such as CNTs makes them ideal for the construction of numerous components (De Volder et al. 2013). Moreover, a variety of NMs are utilised in a number of personal care products including zinc oxide (ZnO) and TiO_2 in sun cream, in addition to products such as moisturiser, foundation and hair colouring (Keller et al. 2014). Zero-valent metals, such as silver (Ag), gold (Au), and iron are commonly used for medical and environmental applications as catalysts of reactive species (Zhang 2003; Klaine et al. 2018). The production volumes and potential applications of NMs influence the risk of toxicity and environmental exposure. For example, carbon-based NM manufacturing rose from 1000 to 5000 metric tons between 2008 and 2015 (Jankovic and Plata 2019). To mitigate the risk associated with the growth of the NM industry, regulations such as REACH in Europe and TSCA in the United States have limited the direct application of NMs for environmental purposes (Royal Society/Royal Academy of Engineering 2004; Jankovic and Plata 2019).

The primary releases and subsequent inadvertent exposures to NMs occur by way of manufacturing, end-use, and disposal (Lead et al. 2018). With the high variability in production volumes and exposure sources, the expansion of the nanotechnology industry has given rise to nanotoxicology, which is a new sub-discipline aimed at understanding NM toxicology, fate, and behaviour and used to assess the human health and environmental effects of NMs. Donaldson et al. (2004) initially proposed the formation of this subcategory to, "…Address the gaps in knowledge and to specifically address special problems to be caused by nanoparticles." The 'special problems' to which Donaldson refers are the unique physico-chemical properties possessed by NMs that give them different properties compared with dissolved or larger scale particles of the same composition. Most notably, NMs have a

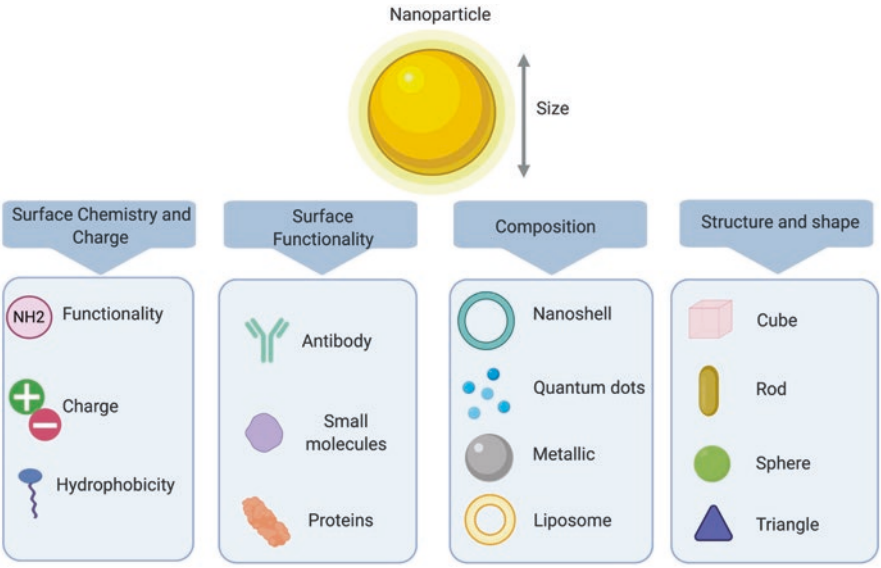


Fig. 1 Example nanomaterial physico-chemical characteristics – size, surface chemistry, charge functionality, composition and surface ligands. (Adapted from Burgum et al. 2018)

high specific surface area (SSA) and surface energy along with undercoordinated bonds which increase the unpredictability of toxicological endpoints and overall uncertainty in risk to biological and ecological systems (Sager et al. 2008; Gałyńska and Persson 2013).

Nanohazard to Human Health

Introduction

The materials evolving from the nanotechnology industry possess fundamental properties very different to their bulk counterparts. In medicine, for example, the specific surface area of these NMs offer unique bioavailabilities that can be targeted to specific sites in the human body (Burgum et al. 2018). With continued increases in usage and development of these NMs there is an inevitable, potentially significant increase in human exposure. While NM physico-chemical characteristics offer great potential in the development of new technologies, the same attributes cause concern towards potential human health hazards. This behaviour is due to the ultra-small nature that gives NMs the potential to be highly reactive within a biological environment. Moreover, this ultra-small size coupled with the geometry of NMs can result in an increased likelihood of the material entering the human body, translocating to different regions other than the portal of entry and promoting adverse interactions at the organ, tissue, and cellular levels.

Human Exposure

The population most at risk of NM exposure is the nanotechnology workforce, i.e., those responsible for synthesising the materials, and who are therefore subject to routine NM exposure, i.e., long-term, possibly low dose (chronic) exposures through daily handling of NMs. This risk is in addition to that of accidental one-off, high dose acute exposures, e.g., during cleaning operations (Ramachandran 2016). Exposure risk according to portal of entry into the body, e.g., dermal, ingestion and inhalation for the nanotechnology workforce during the production process are outlined in Table 2. NM exposure to the general public will likely be lower, through use of NM-containing consumer products or environmental exposure that may lead to low dose, long term exposure on a daily basis. In contrast, individuals exposed to NMs due to medical applications would be subject to short term, controlled exposure in the form of medical imaging and drug delivery systems via intravenous exposure (Nalwa 2014). NM exposure for medical applications will vary depending on the treatment required, varying from a one-off dose for medical imaging to extensive long-term treatment of a chronic condition (Barrow et al. 2017; Patra et al. 2018). Humans are most likely to be exposed to high production NMs (listed in Table 1) and are consequently a primary focus of nano-safety studies (Jankovic and Plata 2019).

There are a limited number of epidemiology studies that have assessed the effect of NM exposure on humans. Those that are available are primarily focused on the nanotechnology workforce. For example, Shvedova et al. (2016) assessed the gene expression profiles of workers having direct contact with multi-walled CNT (MWCNT) aerosols, with an estimated exposure concentration of 14.42 μg m^{-3} within the worker's breathing zone, for at least 6 months. The study revealed that MWCNT exposure resulted in up-regulation of genes involved in a pro-inflammatory response (e.g. IL-6, CSF2, IL-8) indicating potential for the material to cause

Table 2 The potential risks of inhalation, dermal and gastrointestinal tract entry into the body following occupational exposure to NMs during various synthesis processes (http://ec.europa.eu/health/ph_risk)

Synthesis process	Particle formation	Inhalation risks	Dermal/ingestion risks
Gas phase	In air	Reactor leakage Product recovery Post-recovery processing and packaging	Airborne workplace contamination Product handling Plant cleaning/maintenance
Vapour phase	On substrate	Product recovery Post-recovery processing and packaging	Dry workplace contamination Product handling Plant cleaning/maintenance
Colloidal/attrition	Liquid suspension	Product drying Processing/spillage	Workplace spillage/contamination Product handling Plant cleaning/maintenance

pulmonary and cardiovascular complications in humans. A larger epidemiological study recruited 227 workers that physically handled NMs and 137 that did not handle NMs (Liou et al. 2012). The investigation highlighted that workers who handled NMs had decreased levels of antioxidant enzymes superoxide dismutase (SOD) and Glutathione peroxidase (GPX) compared to workers that did not handle the material, thus signifying that cellular oxidative stress increases when working with NM exposure. Wu et al. (2014) undertook a study to measure levels of fractional exhaled nitric oxide (FENO) in 241 workers handling nano-TiO_2. All of the workers investigated had increased FENO levels compared to the control group, showing that continued exposure to aerosolised TiO_2 over time periods up to 5 hours, up to 8 times per week (exposure concentrations not calculated) could potentially result in persistent lung inflammation. These two studies highlight that the nanotechnology industry is beginning to understand the potential risks NMs pose in an occupational setting (Schulte et al. 2019). However, there is a significant need for longitudinal epidemiological investigations with clear exposure characterisations of NMs to more comprehensively understand their potential adverse health effects (Schulte et al. 2019). With this in mind it should be noted that the vast majority of nanotoxicology studies are animal or in vitro based, and conclusions or hypothesises on NM health risk are based on these data rather than human and epidemiological studies to date.

Inhalation

NM entry into the body via the respiratory tract is widely deemed to be a primary route of entry into the human body following occupational exposure (Oberdörster et al. 2005; Geiser and Kreyling 2010). Aerodynamic size is key when considering where an inhaled material will deposit in the respiratory tract, which includes the extra thoracic, upper bronchial, lower bronchial, or the alveolar regions. In simple terms the distance a material is able to penetrate into the lung by diffusional transport is increased with decreasing particle size (Heyder 2004). The ultra-small size of NMs (<100 nm) suggests that a large fraction of them will be deposited within the alveolar region, presuming there is no increase in primary particle size due to agglomeration. The mechanism of deposition is also determined by size, for example a material of ~1 μm in diameter will undergo gravitational sedimentation and inertial impaction, whereas below 100 nm diffusional deposition is the major mechanism (Tsuda et al. 2013).

In vivo studies have confirmed that Ag NPs of 15 nm diameter were found in 3.5-fold greater numbers in the rat alveolus compared to 410 nm Ag NPs (Braakhuis et al. 2014). In silico models have further supported this concept. For instance, application of a multiple-path particle dosimetry model to the nasal inhalation of a 100 nm NM at a concentration of 1 μg m^{-3} in humans showed the greatest deposition to be in the alveolar region of the lung in comparison to a 1 μm particle which deposits mostly in the head and neck region (Manojkumar et al. 2019). This region of the lung is highly vulnerable to NM retention due to the absence of the

mucocilliary elevator and slow clearance by alveolar macrophages that consequently provides the potential for adverse direct and/or indirect NM-interaction with the alveolar epithelium (Maynard and Downes 2019). Key to alveolar macrophage NM-interaction is the initial influence of lung surfactant which is comprised of phospholipids including dipalmitolphosphatidylcholine (DPPC), proteins (SP-A, SP-B, SP-C and SP-D), and numerous neutral lipids (Veldhuizen and Haagsman 2000). Not only do lung surfactant components potentially alter NM surface chemistry via the formation of a NM-corona, but the opsonization function of SP-A and SP-D enhances the ability of alveolar macrophages to phagocytose a foreign material present in the lung (Ruge et al. 2012). Due to the small size and/or shape of NMs clearance by alveolar macrophages may not be possible, resulting in a higher rate of exposure to alveolar cells. NM shape is also a vital consideration; high aspect ratio NMs such as carbon nanotubes or nanofibers may result in frustrated phagocytosis, as the macrophages are unable to fully entrap the material. The result of frustrated phagocytosis is chronic inflammation in the lung tissue causing an inflammatory cascade, immune cell recruitment, and excessive production of reactive oxygen species (ROS) that can cause tissue injury (Cheresh et al. 2013).

Ingestion

The gastrointestinal tract (GI) offers a route for NM entry into the body following intentional consumption, leaching from food containers, deposition onto food, or secondary exposure from inhalation. The GI tract offers a very large surface area of ~200 m^2 for potential NM interaction, similar to the alveolar region in an adult human. Broadly, the GI tract consists of the oesophagus, stomach, duodenum, small intestine, large intestine, and anal canal. Potential NM interactions within these regions include absorption (which allows translocation to the blood and other organs), interaction with the cells comprising the GI tract, or effects on components such as the mucus layer and the microbiome (Bergin and Witzmann 2013). The gut microbiome has become a key area of study due the potential bactericidal toxicity effects of NMs such as Silver NPs (Li et al. 2019). It should be noted that the number of studies that have focused on assessing the effect of NMs on the GI tract is relatively small in comparison to those focusing on the respiratory tract. It can be argued that this lack of focus is due to the low rate of NM passage through the epithelial barrier that has been recorded to occur *in vivo*, although the strong focus on lung studies is also impart due to the historical development of the nanotoxicology field by lung toxicologists (Munger et al. 2014; Van Der Zande et al. 2012; Kreyling et al. 2017c). However, investigations in this open field are beginning to highlight that even a low level of NM absorption in the gut epithelium can result in heavy accumulation over time, ultimately resulting in potential systemic exposure (Kämpfer et al. 2020; Da Silva et al. 2020). NM absorption may potentially occur along the entire GI tract although due to its thick mucus membrane, surrounding connective tissue, and muscular tissue, absorption is highly unlikely to occur in the stomach (Bergin and Witzmann 2013). The acidic environment of the stomach (pH

1.5–3.5) can cause NM dissolution, resulting in the release of dissolved material or non-soluble derivatives, along with aggregation through surface charge neutralization (Kämpfer et al. 2020). Key to the ability of NM interactions with cells of the GI tract is mucus penetration; typically the sticky network of mucin fibres prevents the penetration of foreign materials by steric obstruction and adhesion (Liu et al. 2015). Trapped material is subsequently removed from the tissue either quickly or within a few hours depending on location with the GI tract. Small, negatively charged NMs have been shown to penetrate through mucus more easily than those that are large and positively charged (Wang et al. 2011). This behaviour is based on the principle that, the smaller the particle, the increased likelihood that it is able to pass through the mucus mesh spacings between mucin fibres. Comparison of mucus penetration by different materials established that NMs such as carbon nanotubes CNTs (~210 nm in length) become trapped by adhesive interactions, whereas ZnO NPs (<50 nm in diameter) rapidly penetrated mucus layers (Jachak et al. 2012). Once the barrier has been penetrated, NMs are able to interact with the GI tract epithelium. For example, within the small intestine the epithelium layer is comprised of goblet cells, enteroendocrine, and microfold (M) cells (which are located over Peyer's patches) embedded in a layer of columnar epithelial cells (Fröhlich and Roblegg 2012). It is understood that interaction and uptake by these different cell types is highly dependent on NM size (Unfried et al. 2007) For example, NMs within the size range of 10–50 nm are able to penetrate the epithelial cells (Powell et al. 2010). Alternatively, NMs within the size range of ~50–200 nm are typically in the uptake range of M cells as the NM maybe able to interact with the mechanism used to traffic endogenous calcium phosphate particles into the Peyer's patch immune cells (Powell et al. 2015; Da Silva et al. 2020).

Dermal Penetration

Although arguably not the most significant route into the body, the skin does certainly offer the largest surface area for potential NM contact. Skin exposure may be the result of deposition of an airborne NM, unintentional contact, or intentional application via NM-containing personal care products. The skin is comprised of three major layers; the epidermis, dermis, and subcutaneous layer. The outer layer of the epidermis is termed the stratum corneum, made up of keratinised dead cells, and is the main barrier against penetration (Proksch et al. 2008). A number of investigations into NM human skin penetration via topical application show penetration no deeper than the stratum corneum. This was shown to be the case when 17 nm TiO_2 and 30 nm ZnO NPs only penetrated the stratum corneum and accumulated in hair follicles (Baroli et al. 2007). However, there is evidence that penetration past this initial skin layer is highly probable. A study of 40 nm polystyrene NP penetration in murine skin models showed entry through the hair follicles, into the surrounding dermis, and ultimate passage to draining lymph nodes (although it was noted that mouse skin is thinner than human) (Vogt et al. 2006). Various studies have also reported the correlation between skin damage and increased permeability.

For example, an ex vivo study of Ag NM human skin penetration showed increased permeability through the stratum corneum in damaged skin compared to intact skin (Larese et al. 2009). Similarly, UV damaged porcine skin exhibited increased, although limited, penetration in comparison with the non-damaged model (Miquel-Jeanjean et al. 2012; Monteiro-Riviere et al. 2011). Like all other portals of entry and modes of toxicity, the potential and degree of skin penetration will be dependent of NM properties. The stratum corneum is abundant in cationic filaggrin, and would therefore be more susceptible to penetration by small, anionic NPs (Jatana and Delouise 2014). Due to the increasing prevalence of NM-enabled cosmetic products and the risk of skin exposure in a workplace scenario, the potential of NMs to cause skin sensitization is also an important consideration. It is well known that conditions such metal allergy, e.g., from jewellery or clothing, are major causes of allergic contact dermatitis, an inflammatory disease categorized as delayed-type hypersensitivity (Yoshihisa and Shimizu 2012). Although the exact mechanism of metal allergy is unknown it is believed that metal ions penetrate the skin, promoting an inflammatory response and ultimately $CD4^{+}$T cell activation that causes a characteristic allergic reaction consisting of skin lesions at the site of contact (Saito et al. 2016). NMs also can induce skin sensitization in a similar manner. For example, the local lymph node assay (LLNA) in rabbits has been utilised to demonstrate the ability of <25 nm TiO_2 NPs to induce skin sensitization after topical skin treatment (Park et al. 2011). In recent years NM skin hazard assessment, particularly when centred around cosmetic product assessment, has had to move away from in vivo techniques such as the LLNA due to the ban on the use of animals in cosmetic testing in the European Union (Regulation (EC) No. 1223/2009). This ban has led to the development and use of in vitro reconstructed skin models, such as EpiDerm™ and Straticell, that represent a first point of contact following exposure of a cosmetic product and for skin sensitisation assays (Evans et al. 2017; Choi et al. 2014; Wills et al. 2016).

Ocular Exposure

Despite being in direct contact with the external environment, NM contact with the eyes is often an overlooked potential hazard (Zhu et al. 2019). The eyeball possesses a series of anatomical barriers that prevent the contact of material with the ocular surface, such as blinking and tear film (Pastore 2019). NMs pose a challenge to this protection as their small size may permit close contact with the ocular surface. Subsequent attachment to the cornea and penetration then allows entry into the posterior of the eye (Xu et al. 2013). Although studies evaluating the risks posed by NMs to the eyes are limited, a number of investigations have been undertaken. For example, the effect of 20 and 80 nm gold NPs on mouse retinas over a 72-hour period demonstrated a significant increase in oxidative stress, as measured by Avidin D staining (Söderstjerna et al. 2014). Moreover, an investigation by Sriram et al. (2012) showed that 22.4 and 42.5 nm Ag NPs increase ROS production in bovine retina cells. A recent review by Zhu et al. (2019) has further highlighted ocular

toxicity studies based on NMs used in industrial and environmental locations. The article also stresses that there is a limited number of nano-safety studies that place emphasis on ocular exposure risk and states that the area of study is relatively neglected in comparison to other points of NM exposure.

Translocation from Portal of Entry

There is substantial evidence to suggest that inhaled NMs are capable of distributing from the exposure site to secondary organ systems which include, but are not limited to, the central nervous, hepatic, renal, and cardiovascular systems (Kermanizadeh et al. 2015). Initially NM translocation was not considered a major issue. However, early in vivo nanotoxicology studies demonstrated translocation of radioactive NPs from the lungs, into the blood, and eventually the brain (Nemmar et al. 2001; Oberdörster et al. 2004). An in vitro assessment of the translocation potential of silicon dioxide (SiO_2) and TiO_2 NPs of varying sizes and charge demonstrated that both materials, regardless of characteristics, could translocate across a human bronchial epithelial barrier constructed on a Transwell membrane (George et al. 2015). However, increased translocation rate correlated with decreased NP size and a negative charge. Neither material disrupted the epithelial barrier integrity, suggesting transcytosis of the internalised NPs as a transport mechanism. A similar model comprised of the same cell line showed that carbon nanotubes substantially disrupt the epithelial barrier during translocation (Derk et al. 2015). This form of penetration has also been demonstrated in vivo. CNT (150–200 nm) inhalation in rats showed that the material translocated through the lung and was eventually transported to various organs (Czarny et al. 2014). Size is clearly a key factor in NM translocation. Studies that use an array of differently sized NMs in the majority of cases identify the smallest NMs as having the greatest translocation potential (Kreyling et al. 2017a, b, d). For instance, comparison of Au NP translocation in rats indicated that the smallest particles (of identical shape and composition) (13 ± 12 nm) were able to translocate out of the lung tissue to the blood, liver, spleen, brain, and testes, whereas larger particles (105 ± 42 nm) were only found in the blood rather than secondary organs (Han et al. 2015). Consideration of the ability of an NM to undergo translocation through the body is vital given that this parameter dictates subsequent toxicity at point of entry, and the potential for multi-organ toxicity (Raftis and Miller 2019).

Biological Impact

Ultimately, the primary interactions of NMs in a biological environment occur at the cellular level and involve cellular structures, surfaces and biochemical components (Rothen-Rutishauser et al. 2019). Nanotoxicology studies typically focus on the evaluation of one or more toxicological endpoints, e.g., cytotoxicity,

pro-inflammation and/or genotoxicity. Information on the ability of a test NM to undergo cellular uptake and its localisation within the cell is key in understanding its toxicological fate. Upon interaction with the cell surface, there is the potential for a NM to enter the cell by a number of mechanisms, e.g., phagocytosis, micropinocytosis, caveolin-dependent endocytosis, clathrin-dependent endocytosis, receptor mediated endocytosis, non-specific endocytosis, and passive diffusion, as shown in Fig. 2(i) (Conner and Schmid 2003). Figure 2(ii) shows an example of the uptake of dextran-coated iron oxide NMs in macrophage-like cells derived from the THP-1 cell line. It should be noted that NM uptake may not be limited to one of these mechanisms, i.e., multiple different forms of uptake may be involved for a single NM type (Behra et al. 2013). The ability of a NM to undergo cellular uptake will be dependent on its physico-chemical characteristics, along with the changes to these properties adopted by the material in the biological environment. For example, alteration of surface charges and corona formation can occur as a result of proteins and other macromolecules coating the NM surface (Monopoli et al. 2011).

The Oxidative Stress Paradigm and Its Role in Genotoxicity

The oxidative stress paradigm is key in NM toxicology and plays a central role in promoting genotoxicity (Evans et al. 2019b). Briefly, the term oxidative stress refers to a cellular redox imbalance between reactive oxygen species (ROS), e.g., super oxides ($O_2^{\bullet -}$), hydroxyl radicals ($^{\bullet}OH$), and antioxidants (e.g. SOD), which in a cell's natural state is maintained in homeostasis (Zhang et al. 2016). Many NMs are capable of interacting with oxygen-containing molecules, causing the formation of ROS. For example, ions released from transition metals can react with hydrogen peroxide via Fenton chemistry creating hydroxyl radicals ($^{\bullet}OH$) (Valko et al. 2006):

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)} + {}^{\bullet}OH + OH^{-} \left(\text{where M represents transition metal}\right)$$

The formation of ROS in this manner presents the possibility of inducing DNA damage due to the ability of $^{\bullet}OH$ to readily react with DNA and DNA precursors, resulting in the formation of DNA lesions (Singh et al. 2009). A further example of NM oxidative stress potential is catalysation of ROS formation at the NM surface due to immobilised free bonds. For example, quartz NMs have been shown to promote ROS production due to surface $SiO^{\bullet}$ and $SiO_2^{\bullet}$ moieties (Huang et al. 2010). Oxidative damage to the cellular genetic machinery within a single cell is defined as primary indirect genotoxicity, which is distinct from direct genotoxic mechanisms where an exogenous agent enters the nuclei and directly interferes with the structure and function of DNA. However, evidence of direct induction of genotoxicity by NMs within the literature is limited and is consequently not regarded as a major mechanism of damage (Doak et al. 2012). Aside from primary genotoxicity mechanisms, the ability of a NM to damage DNA can also be mediated by other cell types at the tissue level. This is a prominent mechanism of DNA damage induced by

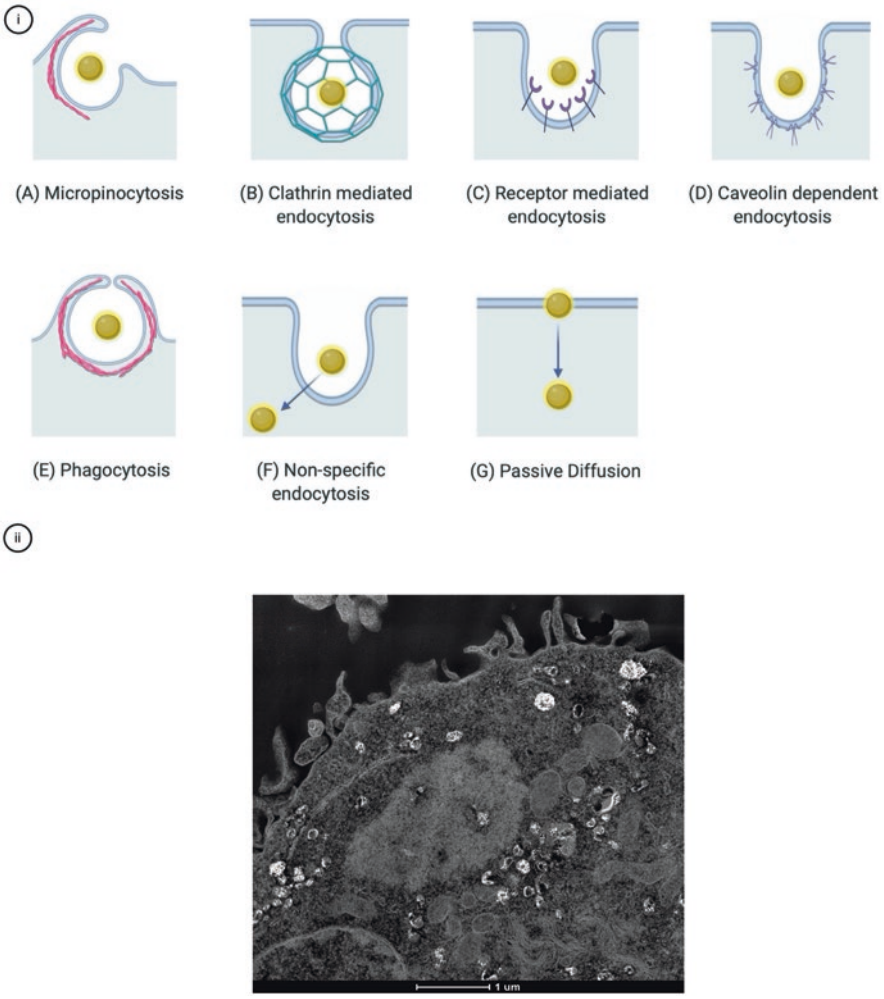


Fig. 2 Active and passive NM cellular uptake mechanisms – (i) Potential mechanisms of NM cellular uptake – (A) Phagocytosis (B) Micropinocytosis (C) Caveolin dependant endocytosis (D) Clathrin mediated endocytosis (E) Receptor mediated endocytosis (F) Non-specific endocytosis (G) Passive diffusion; (ii) Scanning electron microscopy image (STEM) displaying example of iron oxide NM (<10 nm) up take by dTHP-1 cell

NMs, whereby secondary genotoxicity is induced by a chronic pro-inflammatory response that is triggered by immune cells which internalise the invading material. This response subsequently results in oxidatively damaged DNA in surrounding tissues (Evans et al. 2017, 2019a).

Pro-inflammatory Response

The activation of immune cells results in the secretion of various inflammatory mediators, such as cytokines, chemokines, histamines, prostaglandins, ROS, and reactive nitrogen species (RNS). In a balanced biological system, this inflammatory response is vital for pathogen recognition and removal. However, NMs have the potential to disrupt this balance. The potential immunogenicity of a NM will be dependent on a number of factors related to its physico-chemical characteristics, the moieties it presents in regard to cell surfaces interactions, and its ability to undergo cellular uptake (Dobrovolskaia and Mcneil 2016). As discussed in section "Human Exposure", the vast majority of human epidemiology studies that have been undertaken have focused on NM exposure in the workplace with pulmonary inflammation as an outcome. Clear inflammatory markers have been identified in the lungs of workers persistently exposed to aerosolised NMs (Liou et al. 2012). Moreover, a key study by Poland et al. (2008) demonstrated that high aspect ratio NMs behave in a similar manner to asbestos in the lung, causing frustrated phagocytosis and its associated adverse toxicological implications. Consequently, NM immunogenicity potential has been assessed extensively with particular emphasis placed on NMs that are liable to be inhaled, including the NMs highlighted in Table 1. For example, 4–6 nm rutile TiO_2 has been shown to promote immune cell recruitment and cytokine production in the lungs of rats in addition to the onset of cardiac oedema (Nemmar et al. 2008). MWCNT and ZnO NPs can cause increased inflammation and neutrophil recruitment in the lungs of 18-month old mice (Luyts et al. 2018). Moreover, a recent 90-day study by Chu et al. (2019) reaffirmed the ability of carbon black NPs to cause extensive lung and systemic inflammation in rats. Various in vitro NM immunological studies have also been undertaken. For instance, Muller et al. (2010) utilised a lung co-culture model to demonstrate the ability of 20–30 nm TiO_2 NMs to promote an immune response along with increased ROS production. Furthermore, CNT have been shown to promote inflammatory cytokine production and increased ROS in lung epithelial cells in vitro (Fu et al. 2014). Indeed, ROS production and the oxidative stress paradigm is central in most toxicological endpoints associated with NMs.

Summary

The increasing risk of human exposure to NMs has rapidly facilitated the need for hazard and exposure assessment in relation to their effect on human health. While the toxicology of bulk materials is typically influenced by their composition, NMs possess unique physico-chemical properties that in addition to composition determine their ability to enter the human body and their bioreactivity at the organ, tissue, and cellular level. How NMs truly affect human health is not completely understood, but the continually developing field of nanotoxicology is providing evidence into the potential health risks these unique materials pose. In addition to

human health impact, the wider environmental impact of these materials also needs to be considered.

Impacts of NMs on Environmental Health

Exposure to NMs via Discharges and Transformation Processes

At each point in their life cycle, NMs can pass into the environment. NMs can be directly used in the environment for processes such as remediation and can also be discharged via wastewater treatment plants and in industrial effluents from manufacturing sites. From industrial discharges, NMs can enter atmospheric, aquatic, terrestrial, or sedimentary ecosystems (Zhang and Elliott 2006; Biswas and Wu 2005; Selck et al. 2016; Holden et al. 2016; Sun et al., 2017). Possible discharge routes are shown in Fig. 3.

Sources of NM Discharges

While nanotechnology has been viewed as a solution to a number of environmental issues, such as water, soil, and air quality as well as food security (Adeleye et al. 2016; Iavicoli et al. 2017), the complex, energy intensive processes and specialized organic reagents used can outweigh the potential benefits (Pati et al. 2014). The potentially large exposures from production are exacerbated by the uncertainty due to their nano-specific behaviours. A major contributing factor to the environmental footprint of NM production is synthesis yield, with higher yields representing less waste generation and more efficient utilization of resources. Carbon based NMs generally have <33% yield while metal oxides have >90% yield (Jankovic and Plata 2019). Therefore, much of the waste stream from NM production is not NM-laden, but nevertheless can lead to large discharges to the environment as production increases. Additionally, processes and reactants used for synthesizing NMs and any sample handling can leave residual compounds on the NMs, with possible subsequent effects (Oberdörster 2004; Smith et al. 2007; Cheng et al. 2007; Federici et al. 2007; Griffitt et al. 2007; Lee et al. 2007; Oberdörster 2010).

NMs and NM-enabled products are discharged in effluent as solid, liquid, and gaseous wastes (Batley et al. 2013; Ostraat et al. 2013). Unless spilled or used directly into the environment, NM wastes are transmitted through, for example, wastewater treatment facilities, where discharge can occur from sludge to landfill or soil, or from wastewaters discharged to streams after tertiary treatment (Lazareva and Keller 2014). If untreated, NMs can then leach into groundwaters, soil, and surface waters. Sediments, especially marine sediments, are likely to be the final sink for many NMs (Lead et al. 2018). In usage, NMs can slowly be released into the environment, such as with NM-enabled sun creams, textiles, and paints (Nowack

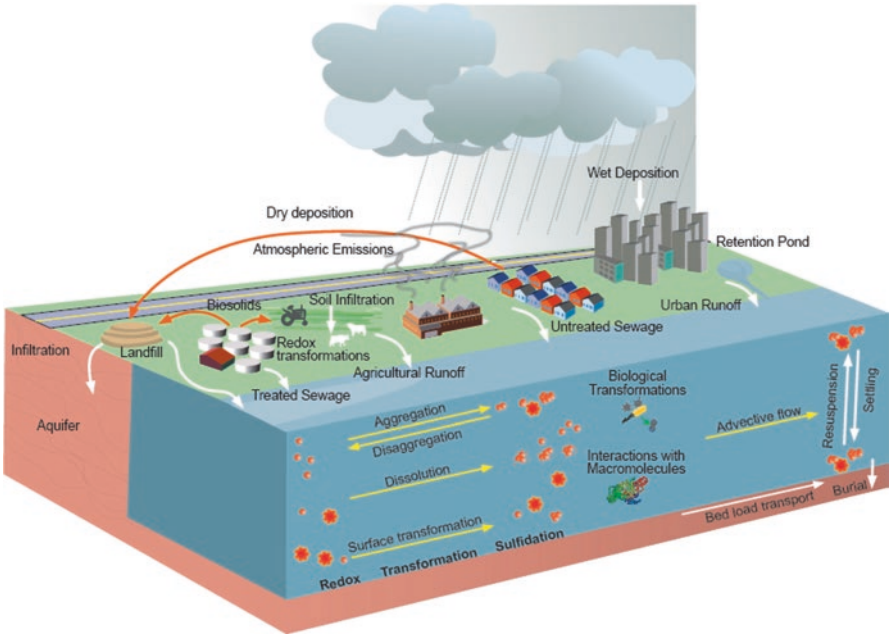


Fig. 3 The major discharge routes of NMs into the environment and potential transformations in the environment. (From Dale et al. 2015)

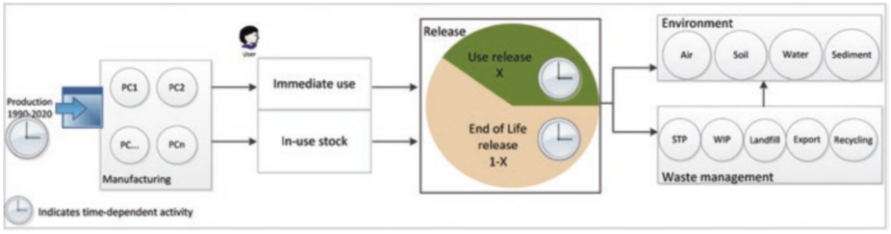


Fig. 4 A schematic of a mass flow analysis (MFA) model. This model uses data on NM production as input, with a percentage allocated to each product category an NM is embedded into. The fractions of NMs in use and in stockpiles, and those of NMs released during use and disposal determine the overall mass of NMs released in the environment. Finally, the concentrations of NMs in environmental and waste management compartments are modelled by analysing the mass of released NMs compared to the size of each compartment. (Wang and Nowack 2018)

et al. 2012). A useful tool for studying the potential environmental exposure to NM-enabled products is the mass flow analysis (MFA) model, shown in Fig. 4 (Wang and Nowack 2018). Fate and behaviour (FB) models have been employed to assess the impact of transformations to NM cores, surface coatings, and intermolecular interactions on environmental fate and behaviour based on inputs from MFA models (Dale et al. 2015; Sun et al. 2017). Some effort has gone into developing a unified approach using both MFA and FB models, which use and produce

complementary datasets (Wang and Nowack 2018). Static MFA is a series of calculations based on the amounts of NMs produced annually, used to estimate the amounts released into the environment (Mueller and Nowack 2008; Gottschalk et al. 2010). Dynamic MFA considers historical production as well as the lifetime of NMs in products to calculate the amount of NMs stored in products and those released into waste infrastructure and environmental compartments (Wang and Nowack 2018). FB models require MFA data for input, and also calculate the compartmentalization of NMs. However, the functions used for these calculations are based on NM physico-chemical properties and the hydrology and geology of the study area (Markus et al. 2017; Ellis et al. 2018; Salieri et al. 2019).

Disposal of NMs occurs when a product reaches its end of life. For instance, disposal of NM-enabled products such as textiles, paints, sun cream and cosmetics, polymers, food packaging, and even food scraps likely transmit the NMs into water treatment plants, landfills or recycling centres (Mitrano et al. 2015). NMs that reach the environment after waste treatment processing are likely to accumulate in sediments (Lowry et al. 2012). NMs are also expected to be released at relatively low concentrations through their use in NM-enabled products (Lead et al. 2018). In terms of discharge, the greatest factors that influence environmental exposure are the volumes and types of NMs that are being used, for which there is scant data (Nowack et al. 2012; Holden et al. 2016). However, there is a clearly increasing trend in volumes of NMs produced and for a worst-case scenario, these gross amounts could be used as a basis for risk assessment. For example, between 2008 and 2015, carbon-based NM production rose from 1000 metric tons to about 5000 metric tons; other heavily produced NMs include SiO_2 (1,000,000 metric tons), TiO_2 (100,000 metric tons), ZnO (50,000 metric tons), zirconium dioxide (50,000 metric tons), aluminium oxide (10,000 metric tons), and CNT (3000 metric tons) (Jankovic and Plata 2019). Based on findings from an MFA model developed by Wang and Nowack (2018), the predicted compartmentalization for five types of NMs is presented in Table 3 for seven regions of Europe.

Environmental Transformations of NMs

Once in the environment, NMs and NM-enabled products undergo chemical, physical, or biological processes that transform the NMs and modify their environmental fate and transport, and biological effects (Nowack et al. 2012; Lowry et al. 2012). Transformations are most likely to occur after a NM-enabled product is disposed of in the environment, rather than during controlled storage, as environmental conditions are more variable (Mitrano et al. 2015). A number of processes that cause NM aging prior to disposal in the environment were determined for silver NM cores embedded in textiles, shown in Table 4. In addition to impacting cores, Mitrano et al., found that the NM coating was also affected by transformations that affected susceptibility to further transformation (2015). These findings highlight the importance of comprehensive understanding of use cases for NMs.

These further transformation, which influences their fate, behaviour, and toxicity (Lowry et al. 2012). Transformations include agglomeration, dissolution, reprecipitation, oxidation, sulfidation, corona formation, and other processes (Nowack et al. 2012; Lowry et al. 2012; Mitrano et al. 2015). For instance, NM agglomeration has been understood within a framework of the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory (Hotze et al. 2010), which describes aggregation for charge stabilized colloids represented as combinations of repulsive and attractive forces (Lead et al. 2018). Agglomeration is frequently observed with NMs at high

Table 3 Lists output from a MFA model for predicted concentrations of NMs in environmental compartments in European regions in 2014 (Wang and Nowack 2018)

NM	Compartment	EU	CE	EE	NE	SE	SEE	CH	Unit
Nano-SiO_2	**STP_{eff}**	65	74	51	48	23	34	12	µg/L
	STP_{sl}	4.4	5.3	2.4	4.6	2.1	3.3	6.8	g/kg
	LW	490	620	450	1500	400	200	–	mg/kg
	IW	490	620	380	470	180	5900	670	mg/kg
	BA	3.4	4.5	2.8	3.1	1.1	25	5.7	g/kg
	FA	4.7	6.1	3.9	4.1	1.5	35	7.9	g/kg
	Air	16	34	8.7	2.6	10	18	48	ng/m3
	NUS	86	170	52	28	68	92	290	µg/kg
	STS	150	390	330	240	240	110	–	mg/kg
	SW	4.3	4.4	2.5	0.22	8.6	11	4.2	µg/L
	Sed	79	79	46	4.1	180	210	75	mg/kg
Nano-CeO_2	**STP_{eff}**	37	44	25	29	19	18	4.8	ng/l
	STP_{sl}	1.8	2.5	0.89	2	1.2	1.3	2.6	mg/kg
	LW	6.5	23	3.3	41	4.4	1.3	–	mg/kg
	IW	8.4	10	8.1	7.8	4.7	150	10	µg/kg
	BA	0.55	0.85	0.66	0.25	0.32	7.1	0.9	mg/kg
	FA	0.77	1.2	0.91	0.36	0.45	9.8	1.2	mg/kg
	Air	42	100	20	6.8	35	43	110	pg/m3
	NUS	42	96	22	14	39	41	120	ng/kg
	STS	60	180	120	110	130	45	–	µg/kg
	SW	2.0	2.6	1.1	0.11	5.1	4.9	1.9	ng/l
	Sed	35	46	19	2.1	95	87	32	µg/kg
Nano-iron oxides	**STP_{eff}**	1.5	1.3	1.2	0.85	0.7	0.56	0.24	µg/L
	STP_{sl}	75	85	44	73	53	45	110	mg/kg
	LW	0.89	1.9	1.03	4.7	1.2	0.35	–	mg/kg
	IW	1	1.2	1.3	0.94	0.67	19	1.4	mg/kg
	BA	22	31	32	11	14	240	42	mg/kg
	FA	32	44	44	16	19	340	59	mg/kg
	Air	0.23	0.53	0.15	0.04	0.25	0.24	0.76	ng/m3
	NUS	0.32	0.67	0.22	0.11	0.33	0.31	1.2	µg/kg
	STS	2.7	6.6	6	4.2	5.8	1.7	–	mg/kg
	SW	86	110	58	4.7	250	180	110	ng/L
	Sed	1.6	2	1.03	0.09	4.4	3.6	2	mg/kg

Table 3 (continued)

NM	Compartment	EU	CE	EE	NE	SE	SEE	CH	Unit
Nano-Al_2O_3	**STP_{eff}**	3.6	5.1	3.5	2.6	2.5	2.5	1.1	µg/L
	STP_{sl}	240	380	160	250	220	240	620	mg/kg
	LW	9.9	20	13	41	16	4.7	–	mg/kg
	IW	9.6	17	11	9.9	6.1	140	21	mg/kg
	BA	93	190	120	64	55	870	300	mg/kg
	FA	130	260	170	88	77	1200	410	mg/kg
	Air	1.2	3.7	0.88	0.21	1.6	1.9	6.5	ng/m3
	NUS	1.3	4.1	1.2	0.51	1.9	2	8.4	ug/kg
	STS	8.2	27	24	14	23	7.9	–	mg/kg
	SW	0.31	0.53	0.22	0.02	1.1	1	0.65	µg/L
	Sed	5.7	9.3	4.1	0.33	19	17	11	mg/kg
Quantum dots	**STP_{eff}**	2.7	3.2	2.4	2.3	1.6	1.6	0.48	pg/L
	STP_{sl}	0.18	0.23	110	0.22	0.14	0.15	0.26	µg/kg
	LW	91	330	57	660	70	22	–	ng/kg
	IW	1.6	1.2	3.4	1.2	1.8	72	0.83	µg/kg
	BA	8	6.1	17	6.1	9.3	260	4.8	µg/kg
	FA	11	8.4	23	8.5	13	350	6.6	µg/kg
	Air	7.9	19	5.1	1.5	8.4	10	22	fg/m3
	NUS	8.4	18	5.8	3	9.1	9.9	27	pg/kg
	STS	6.2	17	16	11	15	5.3	–	ng/kg
	SW	170	180	120	9.6	500	530	150	fg/L
	Sed	3.2	3.2	2.1	0.17	9.1	9.4	2.8	ng/kg

STP sewage treatment plant, *eff* effluent, *sl* sludge, *LW* landfilled waste, *IW* incinerated waste, *BA* bottom ash, *FA* fly ash, *NUS* natural and urban soil, *STS* sludge-treated soil, *SW* surface water, *Sed* sediment, *EU* European Union 27, *CE* Central Europe, *EE* Eastern Europe, *NE* Northern Europe, *SE* Southern Europe, *SEE* Southeastern Europe, *CH* Switzerland

concentrations (tens of mg/L for sterically stabilized NMs), especially in high ionic strength media such as seawater for char charge stabilized NMs, which then settle into the sediment (Doyle et al. 2014; Alabresm et al. 2017). NM concentration, pH, ionic strength, divalent ion concentrations, and NOM concentrations can all influence aggregation behaviour, while surface modification can stabilize NMs (Handy et al. 2008b; Bian et al. 2011; Baalousha et al. 2016). Sedimentation is generally slowed by the presence of natural organic matter (NOM) such as humic substances which form eco-coronas on an NM surface alongside or instead of engineered polymeric coatings (Diegoli et al. 2008; Badawy et al. 2010). Both environmental and engineered coatings affect the colloidal stability, with higher steric stability linked to longer residence times for NMs in a water column (Huynh and Chen 2011; Wang et al. 2016).

Although there is much work on changes in behavior and toxicity due to transformation processes, studies under environmentally relevant conditions are less common. However, improvements in analytical characterization of NMs and NM-enabled products, as well as comprehensive and environmentally relevant

Table 4 Transformation processes for textile-embedded Ag NMs and their causes and effects

Transformation	Cause	Effects
Oxidation	Washing, bleaching	Increased toxicity and decreased effectiveness via dissolution, product leaching as AgCl
	Detergents	Complexation and stabilization
Reduction	Washing	Reduction of Ag ions to new zero-valent particles
Precipitation	Air exposure	Secondary particle formation
	Exposure to digestive fluids	Sulfidation to insoluble Ag/Cl/S complexes
	Washing	Formation of AgCl solids
UV irradiation	Sun exposure	Altered reactivity
	Wastewater treatment	No efficacy in Ag NM removal
Incineration	Disposal	Reduced agglomeration of NMs, production of Ag-laden waste ash
		Degradation of Ag polymer coating and reduction in stability

From Mitrano et al. (2015)

testing protocols, have improved the data obtained from mesocosm studies substantially. Mesocosms are an important study design that enable a more realistic analysis of NM fate and behaviour in the environment than laboratory studies (Lead et al. 2018; Geitner et al. 2020). Mesocosm and laboratory studies sometimes agree but at other times cannot be rationalized. For instance, mesocosm studies on exposure to Ag NMs have suggested that the effects of dissolved and NM formulations of Ag are similar (Colman et al. 2014; Bone et al. 2015), while laboratory studies demonstrate a nano-specific effect dependent on physico-chemical properties, organisms studied, and media (Leclerc and Wilkinson 2014). However, chronic low dose studies with Ag NMs show agreement between mesocosm and laboratory studies (Baker et al. 2016; Merrifield et al. 2017). Further data suggests that sorption of NOM to NMs reduces hazard to organisms by stabilizing NMs in the environment and reducing dissolution, biouptake, and other processes (Mudunkotuwa and Grassian 2015). Previously it was unknown whether a separate and novel risk existed between pristine NMs and NM-enabled products and those that have been weathered by a transformation process (Nowack et al. 2012). However, evidence now suggests that ions released from NMs, NMs in suspension, and agglomerated NMs each exhibit unique environmental behaviours (Lead et al. 2018). Examples of the major transformation processes for NMs are listed in Table 5 (Nowack et al. 2012; Lead et al. 2018).

Obtaining accurate determinations of NM concentrations and extent of transformation in the environment and organisms has been a significant challenge due to the limitations of available analytical techniques (Loosli et al. 2020). However, progress has been made by coupling highly sensitive separation methods to inductively coupled plasma mass spectrometry (ICP-MS) such as asymmetrical flow-field flow fractionation (AF4), or using ICP-MS modes of analysis such as single-cell (SC) and single-particle (SP) workflows, and/or time-of-flight mass (TOF) spectrometry

Table 5 Examples of environmental processes that can lead to NM transformation and influence exposures to organisms (Nowack et al. 2012; Lead et al., 2018)

Process	Type	Example	Reference
Photolysis	Physical/ chemical	Weathering of quantum dots and increase in toxicity	Wiecinski et al. (2013)
Oxidation and reduction	Chemical	Silver oxidation prior to dissolution	Grillet et al. (2013)
Dissolution and precipitation	Physical/ chemical	Silver NMs dissolve into silver ions followed by reprecipitation and ripening	Merrifield et al. (2017)
Adsorption of NMs onto larger particles	Physical/ chemical	Interaction of NMs with external surfaces of organisms followed by uptake	Handy et al. (2008a, b)
Adsorption of NOM and ions onto NMs	Physical or chemical	Ecocorona formation and (commonly) reduction in toxicity	Mudunkotuwa and Grassian (2015)
Combustion	Chemical	Altered CeO_2 NM speciation during sewage sludge incineration	Gogos et al. (2019)
Biodegradation	Biological	Degradation of organic polymer coatings	Kirschling et al. (2011)

(Merrifield et al. 2017; Praetorius et al. 2017; Merrifield et al. 2018; Loosli et al. 2020). SC and SP methods are especially effective in quantifying NMs in biological material, while TOF approaches are able to separate engineered NMs from natural background NMs in environmental media (Gondikas et al. 2018). Further metrological improvements will help to validate MFA and FB modelling approaches which need to be compared with benchmarks for environmental NM concentrations to reduce the uncertainty of the data they generate (Nowack et al. 2012). The uncertainty of the output of MFA and FB models remains high because the probable environmental concentrations and volumes of NMs have uncertainties of several orders of magnitude in some cases, are likely to fluctuate significantly from year to year, and cannot yet be reliably validated on a large scale using current analytical techniques (Jankovic and Plata 2019). The reliability of these models can be improved by using relevant environmental conditions in laboratory experiments and mesocosm studies (Bone et al. 2015; Hansen et al. 2017). Overall, MFA models are useful for estimating and predicting NM releases, and FB models allow researchers to determine the sinks of NMs based on data generated using MFA models. Combining both modelling approaches is promising as a comprehensive tool for fate and transport studies.

The longer a NM is stable in suspension, the greater chance there is for reactions to occur on the NM surface. For instance, sulfidation can occur with metal NMs such as Ag, in environments high in sulfides or low in oxygen (such as anaerobic digesters at wastewater treatment plants – Kim et al. 2010; Kaegi et al. 2011; Lombi et al. 2013). This reaction can influence particle size, charge, and solubility by depositing sulfur atoms onto the NM surface. Depending on NM composition and properties such as size, sulfidation impacts solubility and resulting toxicity to

aquatic organisms (Kaegi et al. 2011). Natural colloids, such as suspended solids, can also change the surface of an NM and influence stability and toxicity (Manciulea et al. 2009; Mudunkotuwa and Grassian 2015; Baalousha et al. 2015; Römer et al. 2016). Agglomeration of dissolved metals into NMs under environmental conditions and sorption of toxic substances have also been observed (Scarano and Morelli 2003; Ferreira et al. 2014).

Terrestrial systems are generally less studied than aquatic systems, though the same principles of colloidal behaviour and challenges in metrology apply to NMs in both media (Burleson et al. 2004; Gimbert et al. 2005; Klaine et al. 2018). Colloid generation, fate, nutrient sequestration, and contaminant transport are influenced by physicochemical properties of the molecules as well as the media, for instance, soil texture, pH, ionic strength, and cation exchange capacity (Quirk and Schofield 1955; Noack et al. 2000; Cornelis et al. 2011; Zhang et al. 2012; Cornelis et al. 2014). As in water, dissolution of ZnO NMs occurs in a pH-dependent manner, and interactions with NOM in the soil reduce toxicity of NMs (Tong et al. 2007; Kasel et al. 2013; Heggelund et al. 2014). To date, inputs of NMs into terrestrial systems have been biosolids applications, which release TiO_2, ZnO, and Ag NMs (Shah et al. 2014). The process of colloid transport through pore spaces in a terrestrial system is shown in Fig. 5 (Cornelis et al. 2014).

Hazard of NMs to Organisms

Environmental transformations, particle size, chemical composition and synthesis method, particle charge, and even shape can impact toxicity of NMs to organisms (Jia et al. 2005; Hawthorne et al. 2012). Benthic crustaceans and mussels uptake NMs and exhibit oxidative DNA damage from exposure (Lovern and Klaper 2006; Zhu et al. 2006; Gagné et al. 2008; Moore et al. 2009). Similarly, earthworms have been shown to exhibit toxic reactions to ZnO NMs in soil (Heggelund et al. 2014). Most ecotoxicological data generated for NMs are for *Daphnia*, algae, bacteria, various species of fish, and some invertebrates such as worms, with investigations into effects on reptiles, birds, and mammals lacking (Lead et al. 2018). A number of toxic effects from NMs have been observed across multiple species. Membrane disruption, changes in ion uptake, cytoskeletal motility, protein function, and generational impacts from NM exposure have been observed in laboratory studies of animals (Park et al. 2003; Soenen et al. 2011; Wu et al. 2013; Yue et al. 2015, 2016; Schultz et al. 2016). Toxicity mechanisms differ between aquatic and terrestrial species; for example, in fish particulate (including NM) uptake occurs through the gills, gastrointestinal tract or skin followed by induction of active transport mechanisms (Geppert et al. 2016). Target organs for NMs are usually similar to bulk or dissolved substances, though the spleen is involved in particulate processing and is particularly affected by NMs in fish (Handy et al. 2011).

The same NM type can have different biological uptake and toxicity behaviour under different conditions. With *Danio rerio* (zebrafish) for example, the rate of Ag

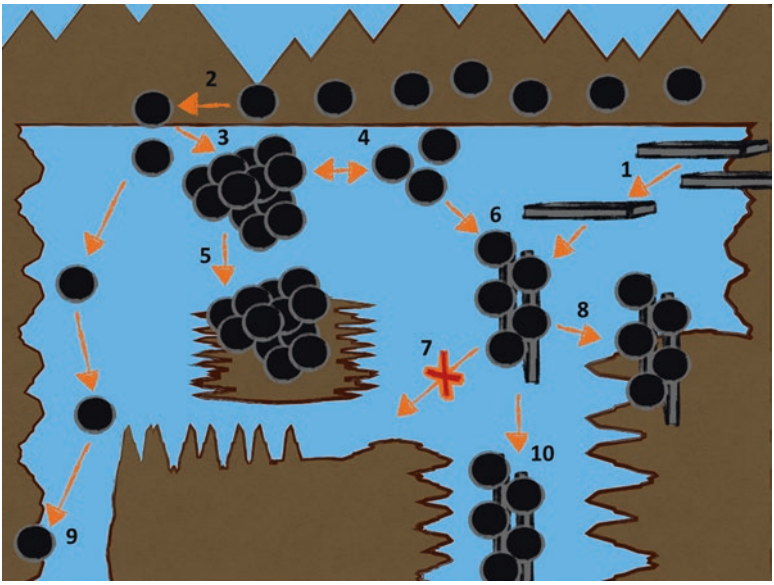


Fig. 5 The fate and transport processes in soil, based on a diagram from Cornelis et al. (2014). **1**: natural colloid formation; **2**: release of NMs from biosolids; **3**: homoaggregation; **4**: fragmentation; **5**: sedimentation; **6**: heteroaggregation; **7**: size exclusion; **8**: straining; **9**: deposition; **10**: convective transport

NM uptake is reduced by the presence of NOM which reduces toxicity (Xiao et al. 2020). Similar behaviour has been observed in other fish as well (Piccapietra et al. 2012; Li et al. 2015; Yue et al. 2017). The sub-lethal effects of NMs in animals may allow for bioaccumulation at higher trophic levels, though the toxicity of chronic ingestion of NM-contaminated food has not yet been established (Judy et al. 2011). In addition, the consumption of food containing NMs alters fish gastrointestinal microbiota, which may be critical in key metabolic processes (Merrifield et al. 2013). Studies on the effects of NMs on zebrafish (*Danio rerio*) have provided basic toxicological data on "no observed effect concentration" (NOEC), "lowest observed effect concentration (LOEC), "median effect concentration" (EC50), and "median lethal concentration" (LC50) for various NMs, and some effort has been placed on developing comprehensive databases of ecotoxicology data for NMs (Juganson et al. 2015). Additionally, zebrafish embryos experience developmental abnormalities from exposure to Au, SiO_2, CdSe/ZnS NMs (King-Heiden et al. 2009; Duan et al. 2013; Kim et al. 2013). Freshwater fish such as rainbow trout (*Oncorhynchus mykiss*) and largemouth bass (*Micropterus salmonoides*) have also been found to be sensitive to Ag and fullerene NM exposures (Oberdörster 2004; Yue et al. 2016).

Several aquatic invertebrates, including snails, crustaceans, and worms have been subjected to ecotoxicology studies based on NM exposures. Oliveira et al., showed that Ag NMs were slightly toxic to the snail *Biomphalaria glabrata* (2019). Castro et al., found that the association of graphene oxide NMs with NOM decreased

the EC50 of the NMs for the crustaceans *Artemia salina* and *Daphnia magna*, suggesting increased toxicity; the same study found that nematodes decreased toxicity to algae, indicating a complex, species dependent interaction with NMs (2018). Lovern and Klaper showed that *Daphnia magna* were also sensitive to TiO_2 and fullerene NMs and found the LC50 to rise significantly when each type of NM was sonicated versus filtered (2006). The estuarine sediment-dwelling lugworm (*Arenicola marina*) also showed gastrointestinal damage with exposure to TiO_2 at a concentration of 1000 μg g^{-1} (Galloway et al. 2010). Gagné showed that freshwater mussels, *Elliption complanata*, suffer immunological damage from CdTe quantum dot NMs (2008). The sea urchin *Paracentrotus lividus* was also shown to have severe developmental defects following nanoplastic exposure (Della Torre et al. 2014). The broad range of affected invertebrates indicates the importance of understanding how NMs behave in the environment.

Microorganisms are also susceptible to the effects of NM exposure. Bacteria such as *Escherichia coli* and *Bacillus subtilis* are affected by fullerenes and quantum dot NMs, as well as antimicrobial NMs such as Ag (Mashino et al. 1999; Kloepfer et al. 2005). Other microorganisms such as algae demonstrate sorption which represents a risk for exposure to fish feeding on them (Leclerc and Wilkinson 2014; Li et al. 2015). Castro et al., found that graphene oxide NMs were also slightly toxic to *Pseudokirchneriella subcapitata* (2018). Several other studies have been performed on algae, which are listed, along with the major fish and invertebrate ecotoxicological data, in Table 6.

The mechanisms of NM localization into specific cellular organelles are not fully understood, though model systems exist for aquatic and terrestrial organisms such as fish, insects, amphibians, and plants (Nations et al. 2011; Priester et al. 2012; Millaku et al. 2013; Bacchetta et al. 2014; Zhao et al. 2015; Minghetti et al. 2017). A key effect of NM exposure is alteration of nitrogen, phosphorus, and carbon cycling (Colman et al. 2013; Schug et al. 2014). For microorganisms, NMs become embedded in extracellular matrices of biofilms and interfere with ion and nutrient uptake (Zhang et al. 2013; Park et al. 2003). NMs that enter cells interact with proteins and organelles such as lysosomes (Linse et al. 2007; Harush-Frenkel et al. 2008; Shemetov et al. 2012; Stern et al. 2012) and mitrochondria (Fröhlich 2013). Smaller NMs such as quantum dots under 9 nm or Au under 39 nm can associate with histones to alter gene expression (Panté and Kann 2002; Nabiev et al. 2007). Larger NMs between 70 and 100 nm can also be incorporated into nuclei during cell division (Lénárt et al. 2003; Chen and von Mikecz 2005).

Environmental risks of NMs and Mitigation Strategies

An NM life cycle can be broken down into production, usage, disposal, and discharges (Holden et al. 2016). Production of NMs may lead to effluent discharge to the air, water, or soil in addition to indirect environmental effects based on energy consumption and synthesis processes employed (Holden et al. 2016). Usage of NMs

Table 6 Selected organisms studied organisms from NMs

Organism	Nanomaterial	NOEC (ug mL^{-1})	EC/ LC50 (ug mL^{-1})	GESAMP rating[a]	Reference
Danio rerio	MXene	50	257.5	Practically non-toxic	Nasrallah et al. (2018a)
	ZnO	<10	13.29	Slightly toxic	Al-Kandari et al. (2019)
	Reduced GO/ TiO_2	<400	748.6	Practically non-toxic	Al-Kandari et al. (2019)
	Magnetic mesoporous SiO_2	1600	>1600	Non-toxic	Nasrallah et al. (2018b)
	Multi-walled CNTs	40	>60	Slightly toxic	Asharani et al. (2008)
	GO	–	>100	Practically non-toxic	Castro et al. (2018)
Daphnia magna	GO	–	>0.58	Highly toxic	Castro et al. (2018)
	Sonicated fullerenes	0.2	7.9	Moderately toxic	Lovern and Klaper (2006)
	Sonicated TiO_2	–	>500	Practically non-toxic	Lovern and Klaper (2006)
	ZrO_2	–	>400	Practically non-toxic	Zaleska-Radziwill and Doskocz (2016)
Pseudokirchneriella subcapitata	GO	–	>66.6	Slightly toxic	Castro et al. (2018)
	ZnO	0.017	0.042–0.068	Very highly toxic	Aruoja et al. (2009) and Franklin et al. (2007)
	TiO2	0.984	5.83	Moderately toxic	Aruoja et al. (2009)
	CuO	0.421	0.71	Highly toxic	Aruoja et al. (2009)
Tetraselmis suecica	ZnO	0.1	3.91	Moderately toxic	Li et al. (2017)
Dunaliella tertiolecta	SiO_2	125	187.77	Practically non-toxic	Manzo et al. (2015)
	TiO_2	7.5	24.1	Slightly toxic	Manzo et al. (2015)
Karenia brevis	TiO_2	–	10.69	Slightly toxic	Li et al. (2015)
Skeletonema costatum	TiO_2	–	7.37	Moderately toxic	Li et al. (2015)

(continued)

Table 6 (continued)

Organism	Nanomaterial	NOEC (ug mL^{-1})	EC/LC50 (ug mL^{-1})	GESAMP rating[a]	Reference
Chlamydomonas reinhardtii	CuO	<100	150.45	Practically non-toxic	Melegari et al. (2013)

NOEC no observed effect concentration, *EC50* median effect concentration, *LC50* median lethal concentration, *GO* graphene oxide.
[a]From GESAMP (2002)

creates another set of pathways into the environment. For example, products such as sun cream that contain TiO_2 in a dispersed form are commonly used at the beach, which washes off directly into the water, while TiO_2 suspended in paint is intended to dry, immobilizing NMs away from environmental discharge (Nowack et al. 2012). Disposal of NMs or NM-enabled products primarily leads to transport through plumbing infrastructure, wastewater treatment plants, and landfills (Holden et al. 2016). Discharges of NMs can occur incidentally through spills or leaks, or through deployment such as in environmental remediation. Especially for remediation applications, understanding the environmental risk of NMs is key prior to regulatory approval (Lead et al. 2018).

To minimize some of the environmental impacts of large-scale NM production, research into green synthesis methods for NMs has been performed (Pati et al. 2014; Jankovic and Plata 2019). For the purposes of comparing the environmental impacts of NM production, a life cycle assessment framework can be employed to weigh the upstream and downstream impacts embodied in a complete product, such as cumulative energy demand of the reactants (embodied energy), carbon emissions, metal depletion, land use, and ecotoxicity (Pati et al. 2014). Additional by-products generated from NM manufacture such as inactive NMs, molecular coatings, and produced wastewater can be discharged directly into the environment in potentially high concentrations (Holden et al. 2016). Incorporation of NMs into products can also create wastes that are then discharged into landfills or wastewater treatment plants (Holden et al. 2016; Lead et al. 2018).

NM surface modifications can improve stability. For example, zero-valent NMs have a highly reactive metal core with an oxidation state of zero, and are highly predisposed to agglomeration. Surface modification provides a balance between increased protection from environmental transformations and reduced reactivity (Klaine et al. 2018). Whether an NM is intended for personal care, environmental use, or other use with potential for accidental spillage or release, these modifications may not last and potential transformations should still be assessed prior to large-scale usage (Lead et al. 2018). Since there can be undesired effects when using synthetic reactants; chemicals generally regarded as safe, such as citrate, polyvinylpyrrolidone, or NOM are competitive alternatives to surfactants and other coatings used to improve environmental stability, since they tend to have higher bioavailability and uptake, lower toxicity, and are more readily biodegradable

(Angel et al. 2013; Pati et al. 2014). While other aspects of a NM production method influence environmental footprint, such as mineral and energy requirements and carbon emissions, these impacts are indirect and are more of a focus for sustainability than ecotoxicology – though still relevant considerations.

Over time, NMs will be cycled through the environment. Prior to this, they may pose a nano-speciifc risk to environmental organisms. Subsequently, they increase total load in the environment, which becomes important for potentially toxic elements and compounds. Currently, studies are limited to a small number of species such as *Daphnia magna*, specific algae and bacteria, and certain fish. In addition, environmental exposure to humans presents a continuing risk (Jankovic and Plata 2019; Holden et al. 2016). The potentially widespread dispersion of NMs into the environment is an issue because they pose a novel risk compared to larger particulates and dissolved counterparts (Lead et al. 2018). The analytical challenges in assessing NM behaviour in real-world situations limits our understanding, although there has been a massive development in knowledge including on the influence of NM properties and transformations on fate, behaviour, and biological effects (Klaine et al. 2018; Nowack et al. 2012; Holden et al. 2016). One drawback to studies performed so far is the lack of long-term exposure data for NMs, which will likely be addressed by integrating newly developed techniques with standardized experimental designs specific to NMs (Lead et al. 2018). Understanding how NMs are weathered in various environments points to the need to assess not only pristine NMs, but NM-enabled products and environmentally transformed NMs as well (Nowack et al. 2012).

Conclusions

The development of environmentally benign processes that include thorough analysis of waste production and active product yield, which work in conjunction with modelling frameworks developed to predict environmental concentrations of NMs, has improved understanding of the environmental risks of NMs (Wang and Nowack 2018; Jankovic and Plata 2019). The focused study on environmental transformations has elucidated the physico-chemical properties of NMs which influence environmental risk, and has hastened the development of engineered surface modifications that limit NM environmental footprints as well as the likelihood of environmental transformation (Balasubramanian and Burghard 2005; Angel et al. 2013; Pati et al. 2014; Yin et al. 2015). Developments in analytical techniques help to validate these models and reduce the uncertainty in estimating NM exposures. In addition, ecotoxicological experiments are increasingly being performed under environmentally relevant operational conditions, providing a clearer idea of the compartmentalization of NMs into environmental and biological systems (Geitner et al. 2020). This effort also provides insight into the threshold concentrations that cause a range of divergent effects including apoptotic responses to enhanced growth. Unique processes in aquatic and terrestrial environments have also been shown to

influence the hazard of NMs in their respective systems and highlight some of the challenges in ecotoxicology for each medium. Evidence of uptake mechanisms for NMs has been obtained, with dynamic effects on microorganisms, plants, and animals alike. A key finding has been the localization sites of NMs into specific cellular organelles based on size, composition, and charge, which enables further minimization of the risk of NMs through knowledge-based product engineering.

Chapter Summary

This chapter has provided an overview of the potential human health and environmental impact of NMs. Presently, the research, development, and production of new NMs is moving faster than the generation of data ascertaining to their hazard risk. The future resolution of this issue is imperative in order to identify NM properties that infer health and environmental risks. The plethora of issues and potential toxicological endpoints broadly covered in this chapter are discussed in further detail throughout the chapters of this book.

Acknowledgements The authors would like to acknowledge all members of the In Vitro Toxicology group at Swansea University who contribute to the exhaustive scientific discussions. In particular, we would like to thank Rachel L. Lawrence, PhD for her assistance in the creation of Figs. 1 and 2 using Biorender.com. We are also grateful to all members of the Center for Environmental Nanoscience and Risk.

Conflict of Interest The authors declare no conflict of interest. The authors are entirely responsible for the content of the book chapter and the perspectives portrayed within it.

References

Adeleye AS, Conway JR, Garner K, Huang YX, Su YM, Keller AA. Engineered nanomaterials for water treatment and remediation: costs, benefits, and applicability. Chem Eng J. 2016;286:640–62.

Alabresm A, Mirshahghassemi S, Chandler GT, Decho AW, Lead JR. Use of PVP-coated magnetite nanoparticles to ameliorate oil toxicity to an estuarine meiobenthic copepod and stimulate the growth of oil degrading bacteria. Environ Sci Nano. 2017;4:1859–65.

Al-Jubory AR, Handy RD. Uptake of titanium from TiO_2 nanoparticle exposure in the isolated perfused intestine of rainbow trout: nystatin, vanadate and novel CO_2-sensitive components. Nanotoxicology. 2013;7:1282–301.

Al-Kandari H, Younes N, Al-Jamal O, Zakaria ZZ, Najjar H, Alserr F, Pintus G, Al-Asmakh MA, Abdullah AM, Nasrallah GK. Ecotoxicological assessment of thermally- and hydrogen-reduced graphene oxide/TiO_2 photocatalytic nanocomposites using the zebrafish embryo model. Nano. 2019;9(4) https://doi.org/10.3390/nano9040488.

Angel BM, Batley GE, Jarolimek CV, Rogers NJ. The impact of size on the fate and toxicity of nanoparticulate silver in aquatic systems. Chemosphere. 2013;93:359–65.

Aruoja V, Dubourguier HC, Kasemets K, Kahru A. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Sci Total Environ. 2009;407(4):1461–8.

Asharani PV, Serina NGB, Nurmawati MH, Wu YL, Gong Z, Valiyaveettil S. Impact of multi-walled carbon nanotubes on aquatic species. J Nanosci Nanotechnol. 2008;8(7):3603–9.
Baalousha MA, Arkill KP, Romer I, Palmer RE, Lead JR. Transformations of citrate and Tween coated silver nanoparticles reacted with Na_2S. Sci Total Environ. 2015;502:344–53.
Baalousha M, Cornelis G, Kuhlbusch T, Lynch I, Nickel C, Peijnenburg W, Van Den Brink N. Modeling nanomaterial fate and uptake in the environment: current knowledge and future trends. Environ Sci Nano. 2016;3:323–45.
Bacchetta R, Moschini E, Santo N, Fascio U, Del Giacco L, Freddi S, Camatini M, Mantecca P. Evidence and uptake routes for zinc oxide nanoparticles through the gastrointestinal barrier in *Xenopus laevis*. Nanotoxicology. 2014;8:728–44.
Badawy AME, Luxton TP, Silva RG, Scheckel KG, Suidan MT, Tolaymat TM. Impact of environmental conditions (pH, ionic strength, and electrolyte type) on the surface charge and aggregation of silver nanoparticles suspensions. Environ Sci Technol. 2010;44:1260–6.
Baker LF, King RS, Unrine JM, Castellon BT, Lowry GV, Matson CW. Press or pulse exposures determine the environmental fate of cerium nanoparticles in stream mesocosms. Environ Toxicol Chem. 2016;35:1213–23.
Balasubramanian K, Burghard M. Chemically functionalized carbon nanotubes. Small. 2005;1:180–92.
Baraton MI. Synthesis, functionalization and surface treatment of nanoparticles: nanoparticles from mechanical action. Valencia: American Science; 2002. Print
Baroli B, Ennas MG, Loffredo F, Isola M, Pinna R, Lopez-Quintela MA. Penetration of metallic nanoparticles in human full-thickness skin. J Investig Dermatol. 2007;127:1701–12.
Barrow M, Taylor A, Fuentes-Caparrós AM, Sharkey J, Daniels LM, Mandal P, Park BK, Murray P, Rosseinsky MJ, Adams DJ. SPIONs for cell labelling and tracking using MRI: magnetite or maghemite? Biomater Sci. 2017;6:101–6.
Batley GE, Kirby JK, McLaughlin MJ. Fate and risks of nanomaterials in aquatic and terrestrial environments. Acc Chem Res. 2013;46(3):854–62.
Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The History of Nanoscience and Nanotechnology: FromChemical-Physical Applications to Nanomedicine. Molecules. 2019;25(1):112.
Behra R, Sigg L, Clift MJD, Herzog F, Minghetti M, Johnston B, Petri-Fink A, Rothen-Rutishauser B. Bioavailability of silver nanoparticles and ions: from a chemical and biochemical perspective. J R Soc Interface. 2013;10:20130396.
Bergin IL, Witzmann FA. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. Int J Biomed Nanosci Nanotechnol. 2013;3 https://doi.org/10.1504/IJBNN.2013.054515.
Beyda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. Molecules. 2020;25(112) https://doi.org/10.3390/molecules25010112.
Bian S-W, Mudunkotuwa IA, Rupasinghe T, Grassian VH. Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir. 2011;27:6059–68.
Biswas P, Wu P. Nanoparticles and the environment. J Air Waste Manage Assoc. 2005;55:708–46.
Bone AJ, Matson CW, Colman BP, Yang X, Meyer JN, Di Giulio RT. Silver nanoparticle toxicity to Atlantic killifish (*Fundulus heteroclitus*) and *Caenorhabditis elegans*: a comparison of mesocosm, microcosm, and conventional laboratory studies. Environ Toxicol Chem. 2015;34:275–82.
Braakhuis HM, Gosens I, Krystek P, Boere JA, Cassee FR, Fokkens PH, Post JA, Van Loveren H, Park MV. Particle size dependent deposition and pulmonary inflammation after short-term inhalation of silver nanoparticles. Part Fibre Toxicol. 2014;11:1–16.
Buffle J, van Leeuwen HP. Environmental particles, vol. 1. Lewis Publishers; 1992.
Buffle J, van Leeuwen HP. Environmental particles, vol. 2. Lewis Publishers; 1993.
Burgum MJ, Evans SJ, Jenkins GJ, Doak SH, Clift MJD. Chapter 10 – Considerations for the human health implications of anotheranostics. In: CONDE J, editor. Handbook of nanomaterials for cancer theranostics. Elsevier; 2018.

Burleson DJ, Driessen MD, Penn RL. On the characterisation of environmental nanoparticles. J Environ Sci Health Part A Tox Hazard Subst Environ Eng. 2004;39:2707–53.
Cameron FK. Soil colloids and the soil solution. J Phys Chem. 1915;19:1–13.
Castro VL, Clemente Z, Jonsson C, Silva M, Vallim JH, de Medeiros AMZ, Martinez DST. Nanoecotoxicity assessment of graphene oxide and its relationship with humic acid. Environ Toxicol Chem. 2018;37(7):1998–2012.
Chaturvedi S, Dave PN, Shah NK. Applications of nano-catalyst in new era. J Saudi Chem Soc. 2012;16(3):307–25.
Chen M, von Mikecz A. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles. Exp Cell Res. 2005;305:51–62.
Cheng J, Flahaut E, Cheng SH. Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos. Environ Toxicol Chem. 2007;26:708–16.
Cheresh P, Kim S-J, Tulasiram S, Kamp DW. Oxidative stress and pulmonary fibrosis. Biochim Biophys Acta. 2013;1832:1028–40.
Choi J, Kim H, Choi J, Oh SM, Park J, Park K. Skin corrosion and irritation test of sunscreen nanoparticles using reconstructed 3D human skin model. Environ Health Toxicol. 2014;29:e2014004.
Chu C, Zhou L, Xie H, Pei Z, Zhang M, Wu M, Zhang S, Wang L, Zhao C, Shi L, Zhang N, Niu Y, Zheng Y, Zhang R. Pulmonary toxicities from a 90-day chronic inhalation study with carbon black nanoparticles in rats related to the systemical immune effects. Int J Nanomedicine. 2019;14:2995–3013.
Colman BP, Arnaout CL, Anciaux S, Gunsch CK, Hochella MF, Kim B, Lowry GV, McGill BM, Reinsch BC, Richardson CJ, Unrine JM, Wright JP, Yin LY, Bernhardt ES. Low concentrations of silver nanoparticles in biosolids cause adverse ecosystem responses under realistic field scenario. PLoS One. 2013;8:10.
Colman BP, Espinasse B, Richardson CJ, Matson CW, Lowry GV, Hunt DE, Wiesner MR, Bernhardt ES. Emerging contaminant or an old toxin in disguise? Silver nanoparticle impacts on ecosystems. Environ Sci Technol. 2014;48:5229–36.
Conner SD, Schmid SL. Regulated portals of entry into the cell. Nature. 2003;422:37–44.
Cornelis G, Ryan B, McLaughlin MJ, Kirby JK, Beak D, Chittleborough D. Solubility and batch retention of CeO_2 nanoparticles in soils. Environ Sci Technol. 2011;45:2777–82.
Cornelis G, Hund-Rinke K, Kuhlbusch T, Van den Brink N, Nickel C. Fate and bioavailability of engineered nanoparticles in soils: a review. Crit Rev Environ Sci Technol. 2014;44:2720–64.
Cortajarena AL, Ortega D, Ocampo SM, Gonzalez-García A, Couleaud P, Miranda R, Belda-Iniesta C, Ayuso-Sacido A. Engineering iron oxide nanoparticles for clinical settings. Nanobiomedicine. 2014;1(2) https://doi.org/10.5772/58841.
Croteau M-N, Dybowska AD, Luoma SN, Valsami-Jones E. A novel approach reveals that zinc oxide nanoparticles are bioavailable and toxic after dietary exposures. Nanotoxicology. 2011;5:79–90.
Croteau M-N, Misra SK, Luoma SN, Valsami-Jones E. Bioaccumulation and toxicity of CuO nanoparticles by a freshwater invertebrate after waterborne and dietborne exposures. Environ Sci Technol. 2014;48:10929–37.
Czarny B, Georgin D, Berthon F, Plastow G, Pinault M, Patriache G, Thuleau A, L'Hermite MM, Taran F, Dive V. Carbon nanotube translocation to distant organs after pulmonary exposure: insights from in situ ^{14}C-radiolabeling and tissue radioimaging. ACS Nano. 2014;8(6):5715–24.
Da Silva AB, Miniter M, Thom W, Hewitt RE, Wills J, Jugdaohsingh R, Powell JJ. Gastrointestinal absorption and toxicity of nanoparticles and microparticles: myth, reality and pitfalls explored through titanium dioxide. Curr Opin Toxicol. 2020;19:112–20.
Dale AL, Casman EA, Lowry GV, Lead JR, Viparelli E, Baalousha M. Modeling nanomaterial environmental fate in aquatic systems. Environ Sci Technol. 2015;49(5):2587–93.
De Volder MFL, Tawfick SH, Baughman RH, Hart JA. Carbon nanotubes: present and future applications. Science. 2013;339:535–9.
Della Torre C, Bergami E, Salvati A, Faleri C, Cirino P, Dawson KA, Corsi I. Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos Paracentrotus lividus. Environ Sci Technol. 2014;48(20):12302–11.

Derk R, Davidson DC, Manke A, Stueckle TA, Rojanasakul Y, Wang L. Potential in vitro model for testing the effect of exposure to nanoparticles on the lung alveolar epithelial barrier. Sens Bio-Sens Res. 2015;3:38–45.

Diegoli S, Manciulea AL, Begum S, Jones IP, Lead JR, Preece JA. Interaction between manufactured gold nanoparticles and naturally occurring organic macromolecules. Sci Total Environ. 2008;402:51–61.

Doak SH, Manshian B, Jenkins GJS, Singh N. In vitro genotoxicity testing strategy for nanomaterials and the adaptation of current OECD guidelines. Mutat Res Genet Toxicol Environ Mutagen. 2012;745:104–11.

Dobrovolskaia MA, Mcneil SE. Immunological properties of engineered nanomaterials: an introduction. In: Handbook of immunological properties of engineered nanomaterials: volume 1: key considerations for nanoparticle characterization prior to immunotoxicity studies. World Scientific; 2016.

Donaldson K, Stone V, Tran CL, Kreyling W, Borm PJA. Nanotoxicology. Occup Environ Med. 2004;61:727–8.

Doyle JJ, Palumbo V, Huey BD, Ward JE. Behavior of titanium dioxide nanoparticles in three aqueous media samples: agglomeration and implications for benthic deposition. Water Air Soil Pollut. 2014;225(9) https://doi.org/10.1007/s11270-014-2106-7.

Duan J, Yu Y, Li Y, Yu Y, Sun Z. Cardiovascular toxicity evaluation of silica nanoparticles in endothelial cells and zebrafish model. Biomaterials. 2013;34:5853–62.

Ellis LJA, Baalousha M, Valsami-Jones E, Lead JR. Seasonal variability of natural water chemistry affects the fate and behaviour of silver nanoparticles. Chemosphere. 2018;191:616–25.

Evans SJ, Clift MJD, Singh N, De Oliveira MJ, Burgum M, Wills JW, Wilkinson TS, Jenkins GJ, Doak SH. Critical review of the current and future challenges associated with advanced in vitro systems towards the study of nanoparticle (secondary) genotoxicity. Mutagenesis. 2017;4:233–41.

Evans SJ, Jenkins GJ, Doak SH, Clift MJD. Cellular defense mechanisms following nanomaterial exposure: a focus on oxidative stress and cytotoxicity. In: Biological Responses to Nanoscale Particles. Springer; 2019a.

Evans SJ, Clift MJD, Singh N, Wills JW, Hondow N, Wilkinson TS, Burgum MJ, Brown AP, Jenkins GJ, Doak SH. In vitro detection of in vitro secondary mechanisms of genotoxicity induced by engineered nanomaterials. Part Fibre Toxicol. 2019b;16:1–14.

Federici G, Shaw BJ, Handy RH. Toxicity of titanium dioxide to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. Aquat Toxicol. 2007;84:415–30.

Ferreira JLR, Lonné MN, Franca TA, Maximilla NR, Lugokenski TH, Costa PG, Fillmann G, Soares FAA, Fernando R, Monserrat JM. Co-exposure of the organic nanomaterial fullerene C_{60} with benzo[a]pyrene in *Danio rerio* (zebrafish) hepatocytes: evidence of toxicological interactions. Aquat Toxicol. 2014;147:76–83.

Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility. Environ Sci Technol. 2007;41:8484–90.

Fröhlich E. Cellular targets and mechanisms in the cytotoxic action of non-biodegradable engineered nanoparticles. Curr Drug Metab. 2013;14:976–88.

Fröhlich E, Roblegg E. Models for oral uptake of nanoparticles in consumer products. Toxicology. 2012;291:10–7.

Fu PP, Xia Q, Hwang H-M, Ray PC, Yu H. Mechanisms of nanotoxicity: generation of reactive oxygen species. J Food Drug Anal. 2014;22:64–75.

Gagné F, Auclair J, Turcotte P, Fournier M, Gagnona C, Sauve S, Blaise C. Ecotoxicity of CdTe quantum dots to freshwater mussels: impacts on immune system, oxidative stress and genotoxicity. Aquat Toxicol. 2008;86:333–40.

Galloway T, Lewis C, Dolciotti I, Johnston BD, Moger J, Regoli F. Sublethal toxicity of nanotitanium dioxide and carbon nanotubes in a sediment dwelling marine polychaete. Environ Pollut. 2010;158:1748–55.

Gałyńska M, Persson P. Emerging polymorphism in nanostructured TiO_2: quantum chemical comparison of anatase, rutile, and brookite clusters. Int J Quantum Chem. 2013;113(24) https://doi.org/10.1002/qua.24522.

Geiser M, Kreyling WG. Deposition and biokinetics of inhaled nanoparticles. Part Fibre Toxicol. 2010;7–2.

Geitner NK, Hendren CO, Cornelis G, Kaegi R, Lead JR, Lowry GV, Lynch I, Nowack B, Petersen E, Bernhardt E, Brown S, Chen W, de Garidel-Thoron C, Hanson J, Harper S, Jones K, von der Kammer F, Kennedy A, Kidd J, Matson C, Metcalfe CD, Pedersen J, Peijnenburg WJGM, Quik JTK, Rodrigues SM, Rose J, Sayre P, Simonin M, Svendsen C, Tanguay R, Tefenkji N, van Teunenbroek T, Thies G, Tian Y, Rice J, Turner A, Liu J, Unrine J, Vance M, White JC, Wiesner MR. Harmonizing across environmental nanomaterial testing media for increased comparability of nanomaterial datasets. Environ Sci Nano. 2020;7:13–36.

George I, Naudin G, Boland S, Mornet S, Contremoulins V, Beugnon K, Martinon L, Lambert O, Baeza-Squiban A. Metallic oxide nanoparticle translocation across the human bronchial epithelial barrier. Nanoscale. 2015;7:4529–44.

Geppert M, Sigg L, Schirmer K. A novel two-compartment barrier model for investigating nanoparticle transport in fish intestinal epithelial cells. Environ Sci Nano. 2016;3:388–95.

Gimbert LJ, Haygarth PM, Beckett R, Worsfold PJ. Comparison of centrifugation and filtration techniques for the size fractionation of colloidal material in soil suspensions using sedimentation field-flow fractionation. Environ Sci Technol. 2005;39:1731–5.

Gogos A, Wielinski J, Voegelin A, Emerich H, Kaegi R. Transformation of cerium dioxide nanoparticles during sewage sludge incineration. Environ Sci Nano. 2019;6:1765–76.

Gondikas A, von der Kammer F, Kaegi R, Borovinskaya O, Neubauer E, Navratilova J, Praetorius A, Cornelis G, Hofmann T. Where is the nano? Analytical approaches for the detection and quantification of TiO_2 engineered nanoparticles in surface waters. Environ Sci Nano. 2018;5(2):313–26.

Gottschalk F, Scholz RW, Nowack B. Probabilistic material flow modeling for assessing the environmental exposure to compounds: methodology and an application to engineered nano-TiO_2 particles. Environ Model Softw. 2010;25:320–32.

Griffitt RJ, Weil R, Hyndman KA, Denslow ND, Powers K, Taylor D, Barber DS. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). Environ Sci Technol. 2007;41:8178–86.

Grillet N, Manchon D, Cottancin E, Bertorelle F, Bonnet C, Broyer M, Lermé J, Pellarin M. Photooxidation of individual silver nanoparticles: a real-time tracking of optical and morphological changes. J Phys Chem C. 2013;117:2274–82.

Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP). The revised GESAMP hazard evaluation procedure for chemical substances carried by ships. GESAMP Rep Stud. 2002;64:33–6.

Han SG, Lee JS, Ahn K, Kim YS, Kim JK, Lee JH, Shin JH, Jeon KS, Cho WS, Song NW, Gulumian M, Shin BS, Yu IJ. Size-dependent clearance of gold nanoparticles from lungs of Sprague-Dawley rats after short-term inhalation exposure. Arch Toxicol. 2015;89:1083–94.

Handy RD, Henry TB, Scown TM, Johnston BD, Tyler CR. Manufactured nanoparticles: their uptake and effects on fish – a mechanistic analysis. Ecotoxicology. 2008a;17:396–409.

Handy RD, Kammer FVD, Lead JR, Hassellöv M, Owen R, Crane M. The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology. 2008b;17:287–314.

Handy RD, Al-Bairuty G, Al-Jubory A, Ramsden CS, Boyle D, Shaw BJ, Henry TB. Effects of manufactured nanomaterials on fishes: a target organ and body systems physiology approach. J Fish Biol. 2011;79:821–53.

Handy RD, Cornelis G, Fernandes T, Tsyusko O, Decho A, Sabo-Attwood T, Metcalfe C, Steevens JA, Klaine SJ, Koelmans AA. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. Environ Toxicol Chem. 2012;31:15–31.

Hansen SF, Hjorth R, Skjolding LM, Bowman DM, Maynard A, Baun A. A critical analysis of the environmental dossiers from the OECD sponsorship programme for the testing of manufactured nanomaterials. Environ Sci Nano. 2017;4:282–91.

Harush-Frenkel O, Rozentur E, Benita S, Altschuler Y. Surface charge of nanoparticles determines their endocytotic and transcytotic pathway in polarized MDCK cells. Biomacromolecules. 2008;9:435–43.

Hawthorne J, Musante C, Sinha SK, White JC. Accumulation and phytotoxicity of engineered nanoparticles to *Cucurbita pepo*. Int J Phytoremediation. 2012;14:429–42.

Heggelund LR, Diez-Ortiz M, Lofts S, Lahive E, Jurkschat K, Wojnarowicz J, Cedergreen N, Spurgeon D, Svendsen C. Soil pH effects on the comparative toxicity of dissolved zinc, non-nano and nano ZnO to the earthworm *Eisenia fetida*. Nanotoxicology. 2014;8:559–72.

Heyder J. Deposition of inhaled particles in the human respiratory tract and consequences for regional targeting in respiratory drug delivery. Proc Am Thorac Soc. 2004;1:315–20.

Holden PA, Gardea-Torresdey JL, Klaessig F, Turco RF, Mortimer M, Hund-Rinke K, Cohen Hubal EA, Avery D, Barceló D, Behra R, Cohen Y, Deydier-Stephan L, Ferguson PL, Fernandes TF, Harthorn BR, Henderson WM, Hoke RA, Hristozov D, Johnston JM, Kane AB, Kapustka L, Keller AA, Lenihan HS, Lovell W, Murphy CJ, Nisbet RM, Petersen EJ, Salinas ER, Scheringer M, Sharma M, Speed DE, Sultan Y, Westerhoff P, White JC, Wiesner MR, Wong EM, Xing B, Horan MS, Godwin HA, Nel AE. Considerations of environmentally relevant test conditions for improved evaluation of ecological hazards of engineered nanomaterials. Environ Sci Technol. 2016;50(12):6124–45.

Hotze EM, Phenrat T, Lowry GV. Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. J Environ Qual. 2010;39:1909–24.

Huang Y-W, Wu C-H, Aronstam RS. Toxicity of transition metal oxide nanoparticles: recent insights from in vitro studies. Materials. 2010;3:4842.

Huynh KA, Chen KL. Aggregation kinetics of citrate and polyvinylpyrrolidone coated silver nanoparticles in monovalent and divalent electrolyte solutions. Environ Sci Technol. 2011;45(13):5564–71.

Iavicoli I, Leso V, Beezhold DH, Shvedova AA. Nanotechnology in agriculture: opportunities, toxicological implications, and occupational risks. Toxicol Appl Pharmacol. 2017;329:96–111.

Jachak A, Lai SK, Hida K, Suk JS, Markovic N, Biswal S, Breysse PN, Hanes J. Transport of metal oxide nanoparticles and single-walled carbon nanotubes in human mucus. Nanotoxicology. 2012;6:614–22.

Jankovic NZ, Plata DL. Engineered nanomaterials in the context of global element cycles. Environ Sci Nano. 2019;6(9):2665–920.

Jatana S, Delouise LA. Understanding engineered nanomaterial skin interactions and the modulatory effects of ultraviolet radiation skin exposure. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2014;6:61–79.

Jia G, Wang H, Yan L, Wang X, Pei J, Yan T, Zhao Y, Guo X. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. Environ Sci Technol. 2005;39:1378–83.

Judy JD, Unrine JM, Bertsch PM. Evidence for biomagnification of gold nanoparticles within a terrestrial food chain. Environ Sci Technol. 2011;45:776–81.

Juganson K, Ivask A, Blinova I, Mortimer M, Kahru A. NanoE-Tox: new and in-depth database concerning ecotoxicity of nanomaterials. Beilstein J Nanotechnol. 2015;6:1788–804.

Kaegi R, Voegelin A, Sinnet B, Zuleeg S, Hagendorfer H, Burkhardt M, Siegrist H. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. Environ Sci Technol. 2011;45:3902–8.

Kämpfer AM, Busch M, Schins RPF. Advanced in vitro testing strategies and models of the intestine for nanosafety research. Chem Res Toxicol. 2020;33:1163–78.

Kasel D, Bradford SA, Simunek J, Putz T, Vereecken H, Klumpp E. Limited transport of functionalized multi-walled carbon nanotubes in two natural soils. Environ Pollut. 2013;180:152–8.

Keller AA, Vosti W, Wang H, Lazareva A. Release of engineered nanomaterials from personal care products throughout their life cycle. J Nanopart Res. 2014;16 https://doi.org/10.1007/s11051-014-2489-9.

Kermanizadeh A, Balharry D, Wallin H, Loft S, Møller P. Nanomaterial translocation-the biokinetics, tissue accumulation, toxicity and fate of materials in secondary organs-a review. Crit Rev Toxicol. 2015;45:837–72.

Kim B, Park CS, Murayama M, Hochella MF. Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. Environ Sci Technol. 2010;44:7509–14.
Kim KT, Zaikova T, Hutchison JE, Tanguay RL. Gold nanoparticles disrupt zebrafish eye development and pigmentation. Toxicol Sci. 2013;133:275–88.
King-Heiden TC, Wiecinski PN, Mangham AN, Metz KM, Nesbit D, Pedersen JA, Hamers RJ, Heideman W, Peterson RE. Quantum dot nanotoxicity assessment using the zebrafish embryo. Environ Sci Technol. 2009;43:1605–11.
Kirschling TL, Golas PL, Unrine JM, Matyjaszewski K, Gregory KB, Lowry GV, Tilton RD. Microbial bioavailability of covalently bound polymer coatings on model engineered nanomaterials. Environ Sci Technol. 2011;45(12):5253–9.
Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem. 2018;27(9):1825–51.
Kloepfer JA, Mielke RE, Nadeau JL. Uptake of CdSe and CdSe/ZnS quantum dots into bacteria via purine-dependent mechanisms. Appl Environ Microbiol. 2005;71:2548–57.
Kreyling WG, Holzwarth U, Schleh C, Kozempel J, Wenk A, Haberl N, Hirn S, Schäffler M, Lipka J, Semmler-Behnke M. Quantitative biokinetics of titanium dioxide nanoparticles after oral application in rats: part 2. Nanotoxicology. 2017a;11:443–53.
Kreyling WG, Holzwarth U, Haberl N, Kozempel J, Hirn S, Wenk A, Schleh C, Schäffler M, Lipka J, Semmler-Behnke M, Gibson N. Quantitative biokinetics of titanium dioxide nanoparticles after intravenous injection in rats: part 1. Nanotoxicology. 2017b:434–442
Kreyling WG, Holzwarth U, Haberl N, Kozempel J, Wenk A, Hirn S, Schleh C, Schäffler M, Lipka J, Semmler-Behnke M, Gibson N. Quantitative biokinetics of titanium dioxide nanoparticles after intratracheal instillation in rats: part 3. Nanotoxicology. 2017c:454–464.
Kreyling WG, Holzwarth U, Schleh C, Kozempel J, Wenk A, Haberl N, Hirn S, Schäffler M, Lipka J, Semmler-Behnke M. Quantitative biokinetics of titanium dioxide nanoparticles after oral application in rats: part 2. Nanotoxicology. 2017d;11:443–53.
Larese FF, D'agostin F, Crosera M, Adami G, Renzi N, Bovenzi M, Maina G. Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicology. 2009;255:33–7.
Lazareva A, Keller AA. Estimating potential life cycle releases of engineered nanomaterials from wastewater treatment plants. ACS Sustain Chem Eng. 2014;2(7):1656–65.
Lead JR, Batley GE, Alvarez PJJ, Croteau M-N, Handy RD, McLaughlin MJ, Judy JD, Schirmer K. Nanomaterials in the environment: behavior, fate, bioavailability, and effects – an updated review. Environ Toxicol Chem. 2018;37(8):2029–63.
Leclerc S, Wilkinson KJ. Bioaccumulation of nanosilver by *Chlamydomonas reinhardtii* – nanoparticle or the free ion? Environ Sci Technol. 2014;48:358–64.
Lee KL, Nallathamby PD, Browning LM, Osgood CJ, Xu X-HN. In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. ACS Nano. 2007;1:133–43.
Lénárt P, Rabut G, Daigle N, Hand AR, Terasaki M, Ellenberg J. Nuclear envelope breakdown in starfish oocytes proceeds by partial NPC disassembly followed by a rapidly spreading fenestration of nuclear membranes. J Cell Biol. 2003;160:1055–68.
Li FM, Liang Z, Zheng X, Zhao W, Wu M, Wang ZY. Toxicity of nano-TiO_2 on algae and the site of reactive oxygen species production. Aquat Toxicol. 2015;158:1–13.
Li J, Schiavo S, Rametta G, Miglietta ML, La Ferrara V, Wu CW, Manzo S. Comparative toxicity of nano ZnO and bulk ZnO towards marine algae *Tetraselmis suecica* and *Phaeodactylum tricornutum*. Environ Sci Pollut Res. 2017;24:6543–53.
Li J, Tang M, Xue Y. Review of the effects of silver nanoparticle exposure on gut bacteria. J Appl Toxicol. 2019;39(1):27–37
Linse S, Cabaleiro-Lago C, Xue WF, Lynch I, Lindman S, Thulin E, Radford SE, Dawson KA. Nucleation of protein fibrillation by nanoparticles. Proc Natl Acad Sci U S A. 2007;104:8691–6.
Liou S-H, Tsou T-C, Wang S-L, Li L-A, Chiang H-C, Li W-F, Lin P-P, Lai C-H, Lee H-L, Lin M-H, Hsu J-H, Chen C-R, Shih T-S, Liao H-Y, Chung Y-T. Epidemiological study of health hazards among workers handling engineered nanomaterials. J Nanopart Res. 2012;14:878.

Liu R, Zhang HY, Ji ZX, Rallo R, Xia T, Chang CH, Nel A, Cohen Y. Development of structure–activity relationship for metal oxide nano- particles. Nanoscale. 2013;5:5644–53.

Liu M, Zhang J, Shan W, Huang Y. Developments of mucus penetrating nanoparticles. Asian J Pharm Sci. 2015;10:275–82.

Liu L, Sun M, Li Q, Zhang H, Yu K, Li M, Zhang C, Cao G, Yuang Y, Zhai H, Chen W, Alvarez PJJ. High-facet-energy CdS nanorods are more reactive but less cytotoxic than lower-facet-energy homologues of similar morphology. Nanotechnol Lett. 2016;16:688–94.

Lombi E, Donner E, Taheri S, Tavakkoli E, Jamting AK, McClure S, Naidu R, Miller BM, Scheckel KG, Vasilev K. Transformation of four silver/silver chloride nanoparticles during anaerobic treatment of wastewater and post-processing of sewage sludge. Environ Pollut. 2013;176:193–7.

Loosli FM, Wang JJ, Sikder M, Afshinnia K, Baalousha M. Analysis of engineered nanomaterials (Ag, CeO_2 and Fe_2O_3) in spiked surface waters at environmentally relevant particle concentrations. Sci Total Environ. 2020;715 https://doi.org/10.1016/j.scitotenv.2020.136927.

Lovern SB, Klaper RD. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C_{60}) nanoparticles. Environ Toxicol Chem. 2006;25:1132–7.

Lowry GV, Gregory KB, Apte SC, Lead JR. Transformations of nanomaterials in the environment. Environ Sci Technol. 2012;46:6893–9.

Luyts K, Van Den Broucke S, Hemmeryckx B, Poels K, Scheers H, Casas L, Vanoirbeek J, Nemery B, Hoet PHM. Nanoparticles in the lungs of old mice: pulmonary inflammation and oxidative stress without procoagulant effects. Sci Total Environ. 2018;644:907–15.

Manciulea A, Baker A, Lead JR. A fluorescence quenching study of the interaction of Suwannee River fulvic acid with iron oxide nanoparticles. Chemosphere. 2009;76:1023–7.

Manojkumar N, Srimuruganandam B, Shiva Nagendra SM. Application of multiple-path particle dosimetry model for quantifying age specified deposition of particulate matter in human airway. Ecotoxicol Environ Saf. 2019;168:241–8.

Manzo S, Buono S, Rametta G, Miglietta M, Schiavo S, Di Francia G. The diverse toxic effect of SiO_2 and TiO_2 nanoparticles toward the marine microalgae *Dunaliella tertiolecta*. Environ Sci Pollut Res. 2015;22(20):15941–51.

Markus AA, Parsons JR, Roex EWM, de Voogt P, Laane RWPM. Modelling the release, transport and fate of engineered nanoparticles in the aquatic environment – a review. Rev Environ Contam Toxicol. 2017;243:53–87.

Mashino T, Okuda K, Hirota T, Hirobe M, Nagano T, Mochizuke M. Inhibition of E. coli growth by fullerene derivatives and inhibition mechanism. Bioorg Med Chem Lett. 1999;9:2959–62.

Maurice PA. Soil science at the nanoscale: a new view of structure, stability, and reactivity. In: Xu J, Huang PM, editors. Molecular environmental soil science at the interfaces in the earth's critical zone. Berlin/Heidelberg: Springer; 2010. https://doi.org/10.1007/978-3-642-05297-2_70.

Maynard RL, Downes N. Chapter 12 – The lung. In: MAYNARD RL, DOWNES N, editors. Anatomy and histology of the laboratory rat in toxicology and biomedical research. Academic; 2019.

Melegari SP, Perreault F, Costa RHR, Popovic R, Matias WG. Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*. Aquat Toxicol. 2013;142:431–40.

Merrifield DL, Shaw BJ, Harper GM, Saoud IP, Davies SJ, Handy RD, Henry TB. Ingestion of metal-nanoparticle contaminated food disrupts endogenous microbiota in zebrafish (*Danio rerio*). Environ Pollut. 2013;174:157–63.

Merrifield RC, Arkill KP, Palmer RE, Lead JR. A high resolution study of dynamic changes of Ce_2O_3 and CeO_2 nanoparticles in complex environmental media. Environ Sci Technol. 2017;51:8014–6.

Merrifield RC, Stephan C, Lead JR. Quantification of Au nanoparticle biouptake and freshwater algae using single cell–ICP-MS. Environ Sci Technol. 2018;52:2271–7.

Millaku A, Drobne D, Torkar M, Novak S, Remskar M, Pipan-Tkalec Z. Use of scanning electron microscopy to monitor nanofibre/cell interaction in digestive epithelial cells. J Hazard Mater. 2013;260:47–52.

Minghetti M, Drieschner C, Bramaz N, Schug H, Schirmer K. A fish intestinal epithelial barrier model established from the rainbow trout (*Oncorhynchus mykiss*) cell line, RTgutGC. Cell Biol Toxicol. 2017;33:539–55.

Miquel-Jeanjean C, Crépel F, Raufast V, Payre B, Datas L, Bessou-Touya S, Duplan H. Penetration study of formulated nanosized titanium dioxide in models of damaged and sun-irradiated skins. Photochem Photobiol. 2012;88:1513–21.

Mitrano DM, Motellier S, Clavaguera S, Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products. Environ Int. 2015;77:132–47.

Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Baldelli Bombelli F, Dawson KA. Physical− chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles. J Am Chem Soc. 2011;133:2525–34.

Monteiro-Riviere NA, Wiench K, Landsiedel R, Schulte S, Inman AO, Riviere JE. Safety evaluation of sunscreen formulations containing titanium dioxide and zinc oxide nanoparticles in UVB sunburned skin: an in vitro and in vivo study. Toxicol Sci. 2011;123:264–80.

Moore MN, Readman JAJ, Readman JW, Lowe DM, Frickers PE, Beesley A. Lysosomal cytotoxicity of carbon nanoparticles in cells of the molluscan immune system: an in vitro study. Nanotoxicology. 2009;3(1):40–5.

Mudunkotuwa IA, Grassian VH. Biological and environmental media control oxide nanoparticle surface composition: the roles of biological components (proteins and amino acids), inorganic oxyanions and humic acid. Environ Sci Nano. 2015;2:429–39.

Mueller NC, Nowack B. Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol. 2008;42:4447–53.

Muller L, Riediker M, Wick P, Mohr M, Gehr P, Rothen-Rutishauser B. Oxidative stress and inflammation response after nanoparticle exposure: differences between human lung cell monocultures and an advanced three-dimensional model of the human epithelial airways. J R Soc Interface. 2010;7:S27–40.

Munger MA, Radwanski P, Hadlock GC, Stoddard G, Shaaban A, Falconer J, Grainger DW, Deering-Rice CE. In vivo human time-exposure study of orally dosed commercial silver nanoparticles. Nanomedicine. 2014;10:1–9.

Nabiev I, Mitchell S, Davies A, Williams Y, Kelleher D, Moore R, Gun'ko YK, Byrne S, Rakovich YP, Donegan JF, Sukhanova A, Conroy J, Cottell D, Gaponik N, Rogach A, Volkov Y. Nonfunctionalized nanocrystals can exploit a cell's active transport machinery delivering them to specific nuclear and cytoplasmic compartments. Nano Lett. 2007;7:3452–61.

Nalwa HS. A special issue on reviews in biomedical applications of nanomaterials, tissue engineering, stem cells, bioimaging, and toxicity. J Biomed Nanotechnol. 2014;10:2421–3.

Nasrallah GK, Al-Asmakh M, Rasool K, Mahmoud KA. Ecotoxicological assessment of $Ti_3C_2T_x$ (MXene) using a zebrafish embryo model. Environ Sci Nano. 2018a;5(4):1002–11.

Nasrallah GK, Zhang Y, Zagho MM, Ismail HM, Al-Khalaf AA, Prieto RM, Albinali KE, Elzatahry AA, Deng YH. A systematic investigation of the bio-toxicity of core-shell magnetic mesoporous silica microspheres using zebrafish model. Microporous Mesoporous Mater. 2018b;265:195–201.

Nations S, Wages M, Cañas JE, Maul J, Theodorakis C, Cobb GP. Acute effects of Fe_2O_3, TiO_2, ZnO and CuO nanomaterials on *Xenopus laevis*. Chemosphere. 2011;83:1053–61.

Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PH, Verbruggen A, Nemery B. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am J Respir Crit Care Med. 2001;164:1665–8.

Nemmar A, Melghit K, Ali BH. The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO_2 nanorods in rats. Exp Biol Med (Maywood). 2008;233:610–9.

Noack AG, Grant CD, Chittleborough DJ. Colloid movement through stable soils of low cation-exchange capacity. Environ Sci Technol. 2000;34:2490–7.

Nowack B, Ranville JF, Diamond S, Gallego-Urrea JA, Metcalfe C, Rose J, Horne N, Koelmans AA, Klaine SJ. Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environ Toxicol Chem. 2012;31(1):50–9.

Oberdörster E. Manufactured nanomaterials (fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth bass. Environ Health Perspect. 2004;112:1058–62.
Oberdörster G. Safety assessment for nanotechnology and nanomedicine: concepts of nanotechnology. J Intern Med. 2010;267(89) https://doi.org/10.1111/j.1365-2796.2009.02187.x.
Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol. 2004;16:437–45.
Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005;113:823–39.
Oliveira-Filho EC, Muniz DHD, de Carvalho EL, Caceres-Velez PR, Fascineli ML, Azevedo RB, Grisolia CK. Effects of AgNPs on the snail *Biomphalaria glabrata*: survival, reproduction and silver accumulation. Toxics. 2019;7(1) https://doi.org/10.3390/toxics7010012.
Ostraat ML, Thornburg JW, Malloy QGJ. Measurement strategies of airborne nanomaterials. Environ Eng Sci. 2013;30(3):126–32.
Panté N, Kann M. Nuclear pore complex is able to transport macromolecules with diameters of about 39 nm. Mol Biol Cell. 2002;13:425–34.
Park KH, Chhowalla M, Iqbal Z, Sesti F. Single-walled carbon nanotubes are a new class of ion channel blockers. J Biol Chem. 2003;278:50212–6.
Park Y-H, Jeong SH, Yi SM, Choi BH, Kim Y-R, Kim I-K, Kim M-K, Son SW. Analysis for the potential of polystyrene and TiO_2 nanoparticles to induce skin irritation, phototoxicity, and sensitization. Toxicol In Vitro. 2011;25:1863–9.
Pastore C. In your eyes. Nat Nanotechnol. 2019;14:306.
Pati P, McGinnis S, Vikesland PJ. Life cycle assessment of "green" nanoparticle synthesis methods. Environ Eng Sci. 2014;31(7) https://doi.org/10.1089/ees.2013.0444.
Patra JK, Das G, Fraceto LF, Campos EVR, Del Pilar Rodriguez-Torres M, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S. Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol. 2018;16:71.
Piccapietra F, Allué CG, Sigg L, Behra R. Intracellular silver accumulation in *Chlamydomonas reinhardtii* upon exposure to carbonate coated silver nanoparticles and silver nitrate. Environ Sci Technol. 2012;46(13):7390–7.
Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WAH, Seaton A, Stone V, Brown S, MacNee W, Donaldson K. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol Lett. 2008;3:423–8.
Powell JJ, Faria N, Thomas-Mckay E, Pele LC. Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract. J Autoimmun. 2010;34:J226–33.
Powell JJ, Thomas-Mckay E, Thoree V, Robertson J, Hewitt RE, Skepper JN, Brown A, Hernandez-Garrido JC, Midgley PA, Gomez-Morilla I. An endogenous nanomineral chaperones luminal antigen and peptidoglycan to intestinal immune cells. Nat Nanotechnol. 2015;10:361–9.
Praetorius A, Gundlach-Graham A, Goldberg E, Fabienke W, Navratilova J, Gondikas A, Kaegi R, Gunther D, Hofmann T, von der Kammer F. Single-particle multi-element fingerprinting (spMEF) using inductively-coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) to identify engineered nanoparticles against the elevated natural background in soils. Environ Sci Nano. 2017;4(2):307–14.
Priester JH, Ge Y, Mielke RE, Horst AM, Moritz SC, Espinosa K, Gelb J, Walker SL, Nisbet RM, An Y-J. Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption. Proc Natl Acad Sci U S A. 2012;109:E2451–6.
Proksch E, Brandner JM, Jensen J-M. The skin: an indispensable barrier. Exp Dermatol. 2008;17:1063–72.
Quirk JP, Schofield RK. The effect of electrolyte concentration on soil permeability. J Soil Sci. 1955;6:163–78.
Raftis JB, Miller MR. Nanoparticle translocation and multi-organ toxicity: a particularly small problem. Nano Today. 2019;26:8–12.
Ramachandran G. Assessing nanoparticle risks to human health. William Andrew; 2016.
Römer I, Wang ZW, Merrifield RC, Palmer RE, Lead J. High resolution STEM-EELS study of silver nanoparticles exposed to light and humic substances. Environ Sci Technol. 2016;50:2183–90.

Rothen-Rutishauser B, Bourquin J, Petri-Fink A. Nanoparticle-cell interactions: overview of uptake, intracellular fate and induction of cell responses. In: Biological Responses to Nanoscale Particles. Springer; 2019.

Royal Society/Royal Academy of Engineering. Nano-science and nanotechnologies: opportunities and uncertainties. Two year review of progress on government actions: joint academies' response to the Council for Science and Technology's call for evidence. RS Policy Document 35/06. London: The Royal Society; 2004.

Ruge CA, Schaefer UF, Herrmann J, Kirch J, Cañadas O, Echaide M, Pérez-Gil J, Casals C, Müller R, Lehr C-M. The interplay of lung surfactant proteins and lipids assimilates the macrophage clearance of nanoparticles. PLoS One. 2012;7:e40775.

Sager TM, Kommineni C, Castranova V. Pulmonary response to intratracheal instillation of ultrafine versus fine titanium dioxide: role of particle surface area. Part Fibre Technol. 2008;5(17) https://doi.org/10.1186/1743-8977-5-17.

Saito M, Arakaki R, Yamada A, Tsunematsu T, Kudo Y, Ishimaru N. Molecular mechanisms of nickel allergy. Int J Mol Sci. 2016;17:202.

Salieri B, Hischier R, Quik JTK, Jolliet O. Fate modelling of nanoparticle releases in LCA: An integrative approach towards "USEtox4Nano". J Clean Prod. 2019;206:701–12.

Scarano G, Morelli E. Properties of phytochelatin-coated CdS nanocrystallites formed in a marine phytoplanktonic alga (*Phaeodactylum tricornutum*, Bohlin) in response to cd. Plant Sci. 2003;165:803–10.

Schlather AE, Gieri P, Robinson M, Centeno SA, Manjavacas A. Nineteenth-century nanotechnology: the plasmonic properties of daguerrotypes. PNAS. 2019;116(28):13791–8.

Schug H, Isaacson C, Sigg L, Ammann A, Schirmer K. Effect of TiO_2 nanoparticles and UV radiation on extracellular enzyme activity of intact heterotrophic biofilms. Environ Sci Technol. 2014;48:11620–8.

Schulte PA, Leso V, Niang M, Iavicoli I. Current state of knowledge on the health effects of engineered nanomaterials in workers: a systematic review of human studies and epidemiological investigations. Scand J Work Environ Health. 2019;45:217–38.

Schultz C, Wamucho A, Tsyusko OV, Unrine JM, Crossley A, Svendsen C, Spurgeon DJ. Multigenerational exposure to silver ions and silver nanoparticles reveals heightened sensitivity and epigenetic memory in *Caenorhabditis elegans*. Proc R Soc B. 2016;283(1832) https://doi.org/10.1098/rspb.2015.2911.

Selck H, Handy RD, Fernandes TF, Klaine SJ, Petersen EJ. Nanomaterials in the aquatic environment: a European Union–United States perspective on the status of ecotoxicity testing, research priorities, and challenges ahead. Environ Toxicol Chem. 2016;35:1055–67.

Shah V, Jones J, Dickman J, Greenman S. Response of soil bacterial community to metal nanoparticles in biosolids. J Hazard Mater. 2014;274:399–403.

Shemetov AA, Nabiev I, Sukhanova A. Molecular interaction of proteins and peptides with nanoparticles. ACS Nano. 2012;6:4585–602.

Shvedova AA, Yanamala N, Kisin ER, Khailullin TO, Birch ME, Fatkhutdinova LM. Integrated analysis of dysregulated ncRNA and mRNA expression profiles in humans exposed to carbon nanotubes. PLoS One. 2016;11(3):e0150628.

Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, Maffeis TG, Wright CJ, Doak SH. NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. Biomaterials. 2009;30:3891–914.

Smith C, Shaw BJ, Handy RH. Toxicity of single walled carbon nanotubes on rainbow trout (*Oncorhyncos mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. Aquat Toxicol. 2007;84:415–30.

Söderstjerna E, Bauer P, Cedervall T, Abdshill H, Johansson F, Johansson UE. Silver and gold nanoparticles exposure to in vitro cultured retina – studies on nanoparticle internalization, apoptosis, oxidative stress, glial- and microglial activity. PLoS One. 2014;9:e105359.

Soenen SJ, Rivera-Gil P, Montenegro J-M, Parak WJ, De Smedt SC, Braeckmans K. Cellular toxicity of inorganic nanoparticles: common aspects and guidelines for improved nanotoxicity evaluation. Nano Today. 2011;6:446–65.

Sriram MI, Kalishwaralal K, Barathmanikanth S, Gurunathani S. Size-based cytotoxicity of silver nanoparticles in bovine retinal endothelial cells. Nanosci Methods. 2012;1:56–77.
Stern ST, Adiseshaiah PP, Crist RM. Autophagy and lysosomal dysfunction as emerging mechanisms of nanomaterial toxicity. Part Fibre Toxicol. 2012;9:20.
Sun TY, Mitrano DM, Bornhöft NA, Scheringer M, Hungerbühler K, Nowack B. Envisioning nano release dynamics in a changing world: using dynamic probabilistic modeling to assess future environmental emissions of engineered nanomaterials. Environ Sci Technol. 2017;51:2854–63.
Tong Z, Bischoff M, Nies L, Applegate B, Turco RF. Impact of fullerene (C_{60}) on a soil microbial community. Environ Sci Technol. 2007;41:2985–91.
Tsuda A, Henry FS, Butler JP. Particle transport and deposition: basic physics of particle kinetics. Compr Physiol. 2013;3:1437–71.
Unfried K, Albrecht C, Klotz L-O, Von Mikecz A, Grether-Beck S, Schins RPF. Cellular responses to nanoparticles: Target structures and mechanism. Nanotoxicology. 2007;1:1:52–71
Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160:1–40.
Van Der Zande M, Vandebriel RJ, Van Doren E, Kramer E, Herrera Rivera Z, Serrano-Rojero CS, Gremmer ER, Mast J, Peters RJ, Hollman PC. Distribution, elimination, and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. ACS Nano. 2012;6:7427–42.
Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF Jr, Rejeski D, Hull MS. Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory. Beilstein J Nanotechnol. 2015;6:1769–80.
Veldhuizen EJ, Haagsman HP. Role of pulmonary surfactant components in surface film formation and dynamics. Biochim Biophys Acta. 2000;1467:255–70.
Vogt A, Combadiere B, Hadam S, Stieler KM, Lademann J, Schaefer H, Autran B, Sterry W, Blume-Peytavi U. 40 nm, but not 750 or 1,500 nm, nanoparticles enter epidermal CD1a+ cells after transcutaneous application on human skin. J Investig Dermatol. 2006;126:1316–22.
Wang Y, Nowack B. Dynamic probabilistic material flow analysis of nano-SiO_2, nano iron oxides, nano-CeO_2, nano-Al_2O_3, and quantum dots in seven European regions. Environ Pollut. 2018;235:589–601.
Wang Y-Y, Lai SK, So C, Schneider C, Cone R, Hanes J. Mucoadhesive nanoparticles may disrupt the protective human mucus barrier by altering its microstructure. PLoS One. 2011;6:e21547.
Wang ZY, Zhang L, Zhao J, Xing BS. Environmental processes and toxicity of metallic nanoparticles in aquatic systems as affected by natural organic matter. Environ Sci Nano. 2016;3(2):240–55.
Wiecinski PN, Metz KM, Heiden TCK, Louis KM, Mangham AN, Hamers RJ, Heideman W, Peterson RE, Pedersen JA. Toxicity of oxidatively degraded quantum dots to developing zebrafish (*Danio rerio*). Environ Sci Technol. 2013;47(16):9132–9.
Wills JW, Hondow N, Thomas AD, Chapman KE, Fish D, Maffeis TG, Penny MW, Brown RA, Jenkins GJ, Brown AP, White PA, Doak SH. Genetic toxicity assessment of engineered nanoparticles using a 3D in vitro skin model (EpiDerm). Part Fibre Toxicol. 2016;13:016–0161.
Wu J, Li X, Yan Y, Hu Y, Zhang Y, Tang Y. Protein adsorption onto nanozeolite: effect of micropore openings. J Colloid Interface Sci. 2013;406:130–8.
Wu WT, Liao HY, Chung YT, Li WF, Tsou TC, Li LA, Lin MH, Ho JJ, Wu TN, Liou SH. Effect of nanoparticles exposure on fractional exhaled nitric oxide (FENO) in workers exposed to nanomaterials. Int J Mol Sci. 2014;15:878–94.
Xiao BW, Wang XL, Yang J, Wang KK, Zhang YQ, Sun BB, Zhang T, Zhu LY. Bioaccumulation kinetics and tissue distribution of silver nanoparticles in zebrafish: the mechanisms and influence of natural organic matter. Ecotoxicol Environ Saf. 2020;194 https://doi.org/10.1016/j.ecoenv.2020.110454.
Xu Q, Kambhampati SP, Kannan RM. Nanotechnology approaches for ocular drug delivery. Middle East Afr J Ophthalmol. 2013;20:26–37.
Yin Y, Shen M, Tan Z, Yu S, Liu J, Jiang G. Particle coating-dependent interaction of molecular weight fractionated natural organic matter: impacts on the aggregation of silver nanoparticles. Environ Sci Technol. 2015;49:6581–9.

Yokoyama T, Huang CC. Nanoparticle technology in the production of functional materials. Kona J. 2005;23:7–17.
Yoshihisa Y, Shimizu T. Metal allergy and systemic contact dermatitis: an overview. Dermatol Res Pract. 2012;2012:749561.
Yue Y, Behra R, Sigg L, Freire PF, Pillai S, Schirmer K. Toxicity of silver nanoparticles to a fish gill cell line: role of medium composition. Nanotoxicology. 2015;9:54–63.
Yue Y, Behra R, Sigg L, Suter MJF, Pillai S, Schirmer K. Silver nanoparticle-protein interactions in intact rainbow trout gill cells. Environ Sci Nano. 2016;3:1174–85.
Yue Y, Li X, Sigg L, Suter MJ, Pillai S, Behra R, Schirmer K. Interaction of silver nanoparticles with algae and fish cells: a side by side comparison. J Nanobiotechnol. 2017;15:16.
Zaleska-Radziwill M, Doskocz N. Ecotoxicity of zirconium oxide nanoparticles in relation to aquatic invertebrates. Desalin Water Treat. 2016;57(3):1443–50.
Zhang W. Nanoscale iron particles for environmental remediation: An overview. J Nanopart Res. 2003;5:323–32.
Zhang W-X, Elliott DW. Applications of iron nanoparticles for groundwater remediation. Remediation. 2006;16:7–21.
Zhang L, Hou L, Wang LL, Kan AT, Chen W, Tomson MB. Transport of fullerene nanoparticles (nC_{60}) in saturated sand and sandy soil: controlling factors and modeling. Environ Sci Technol. 2012;46:7230–8.
Zhang S, Jiang Y, Chen C-S, Creeley D, Schwehr KA, Quigg A, Chin W-C, Santschi PH. Ameliorating effects of extracellular polymeric substances excreted by *Thallassiosira pseudonana* on algal toxicity of CdSe quantum dots. Aquat Toxicol. 2013;126:214–23.
Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, Dong W. ROS and ROS-mediated cellular signaling. Oxidative Med Cell Longev. 2016;2016:18.
Zhao L, Sun Y, Hernandez-Viezcas JA, Hong J, Majumar S, Niu G, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresday JL. Monitoring the environmental effects of CeO_2 and ZnO nanoparticles through the life cycle of corn plants an in situ m-XRF mapping of nutrients in kernels. Environ Sci Technol. 2015;49:2921–8.
Zhu Y, Zhao Q, Li Y, Cal X, Li W. The interaction and toxicity of multi-walled carbon nanotubes with *Stylonychia mytilus*. J Nanosci Nanotechnol. 2006;6:1357–64.
Zhu S, Gong L, Li Y, Xu H, Gu Z, Zhao Y. Safety assessment of nanomaterials to eyes: an important but neglected issue. Adv Sci (Weinheim, Baden-Wurttemberg, Germany). 2019;6:1802289.

The Potential Adverse Effects of Engineered Nanomaterial Exposure to Human Health Following Pulmonary, Oral and Dermal Exposure

Ali Kermanizadeh, Flemming R. Cassee, and Wim de Jong

Contents

Abstract Nanotechnology allows the production of nano-sized particles and fibres with properties that are not evident in the micro-size range. This also has an impact on their uptake, accumulation and excretion in the body as well as their toxic potency and associated health effects. There remians little evidence published as to the unique toxicity of nano-sized particles and fibres. Three main routes of exposure (oral, inhalation and dermal) are described herein, as well as key toxicological observations caused by nano-sized particles and fibres. Albeit that a lot of research has been performed, particular to increase our understanding of acute effects, the field is not yet at the stage to formulate new paradigms that would support the grouping and classification of nanomaterials without the need for rigorous testing of each specific material.

Keywords Toxicity · Oral · Dermal · Inhalation · Nanomaterials

A. Kermanizadeh
University of Derby, School of Human Sciences, Human Sciences Research Centre, Derby, UK

F. R. Cassee (✉)
National Institute for Public Health and the Environment Netherlands, Bilthoven, Netherlands

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands
e-mail: flemming.cassee@rivm.nl

W. de Jong
National Institute for Public Health and the Environment Netherlands, Bilthoven, Netherlands

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_2

Introduction

Nanotechnology finds application in many different areas. For example, objects can be made stronger than normal, and solar cells can have improved efficiencies. Nanotechnology also makes it possible to ensure that medicines are targeted to a specific location in the body where they are needed. Due to these highly promising characteristics, the government and the business community invest a great deal in nanomaterials (NMs), and the technology has become an inseparable part of our society. Although there is not yet a uniform definition of a NM, especially for use in legislation, there is a general consensus that nanoparticles, have at least one dimension <100 nm (ISO TS 80004-1:2015, ISO 80004-2:2015, EU Recommendation). NMs and/or nanoparticles are being incorporated at a rapid rate into new structures, materials and devices with a wide range of commercial applications (e.g. sunscreens as UV filter, paints for scratch resistance, solar cells for energy generation). Since nanoparticles exhibit physicochemical properties strikingly different than fine particles (micrometer range) of the same composition, there is every reason to suspect that nanoparticles could exhibit unique bioactivity. Therefore, the field of nanotoxicology has emerged to evaluate pulmonary and systemic effects of exposure to nanoparticles, determine the dose dependency and time course of these responses, and identify mechanisms of action. Environmental assessment studies indicate that nanoparticles can be aerosolized during weighing, blending, mixing, sonication, and spraying procedures [Maynard et al. 2004; Han et al. 2008; Lee et al. 2010; Stone et al. 2017]. In addition, high levels of aerosolized nanoparticles can be generated during cleaning of ovens used for nanoparticles synthesis [Methner 2008]. Therefore, inhalation is believed to be a primary route of nanoparticle exposure in the workplace or, in case of using spray products like deodorant or sunscreen that poses the highest risk for adverse effects (Riediker et al. 2019; Riebeling et al. 2016). However, the knowledge and understanding the mechanisms of nanotoxicology continues to develop, as reflected by the constant growth in literature in the past decade.

A few studies are now emerging that demonstrate effects of NMs on human health, especially in an occupational setting (Willhite et al. 2016; Gulumian et al. 2016; Riediker et al. 2019). For multi-walled carbon nanotubes (MWCNTs), Lee et al. (2015) investigated workers manufacturing this material and found that while there was no impact on haematology and blood biochemistry, they did see an increase in a range of markers of lipid peroxidation in exhaled breath condensates of workers, including malondialdehyde, 4-hydroxy-2-hexenal and n-hexanal. MWCNTs have also been reported to impact on a range of endpoints in workers exposed for at least 6 months. These endpoints include the targeting of genes associated with the cell cycle regulation, progression and control as well as genes involved in apoptosis and proliferation (Shvedova et al. 2016). The same study also identified targeting of pathways involved in pulmonary and cardiovascular effects, as well as carcinogenic outcomes in humans. Another study followed workers in 14 NM manufacturing and/or application factories in Taiwan for 6 months (Liao et al. 2014).

The NMs made or handled included silver, iron oxide, gold, titanium dioxide, carbon nanotubes or silicon dioxide. The group working with NMs exhibited higher levels of antioxidant enzymes cardiovascular markers than workers handling other materials. In addition, the study also identified that markers of small airway damage and lung function were significantly associated with handling NMs.

Respiratory Response to Pulmonary Exposure

There is still relatively little information available on whether exposure to NMs can lead to health effects. This is partly due to the absence of an overview of companies working with NMs or the lack of information on the presence of NMs in e.g. consumer products. At the workplace, we do know that the likelihood of exposure to NMs depends primarily on the chance of nanoparticles being inhaled. This may, for instance, happen when NMs are handled in powder form or in spray applications (e.g. spray paint), or when processing materials that contain nanoparticles. Apart from workers, consumers can also be exposed via inhalation for instance by using spray products such as deodorants.

Once inhaled, nanoparticles have a high probability of deposition in the lungs (Kuempel et al. 2015). This deposition occurs primarily by diffusion and secondarily by thermophoretic effects in the first few airways of the lung during inhalation and exhalation. Fibres and platelets like graphene that are nano-sized in at least one dimension are also deposited in the lower respiratory tract, mainly by interception due to their small size and elongated shape. Once deposited the chemical composition including surface reactivity and dissolution rates are the driving forces for toxicity. Oxidative stress leading to inflammatory responses is a common biological response that can lead to tissue damage as well as lung cancer including mesothelioma related to biopersistent fibres (Stone et al. 2017). Particles can be translocated to other organs including into the blood where they have been associated with the onset of adverse health outcomes (Kreyling et al. 2013; Kuempel et al. 2015; Geiser and Kreyling 2017; Riediker et al. 2019) (Fig. 1).

There are several theories regarding the toxicity of NM and how it may differ from larger particles in air. Toxicity will depend on the chemical composition, external (crystallinity) and internal structure (open spaces, porosity) and impurities, and particle size/dimensions (Johnston et al. 2000). High particle number, overall large specific surface areas, and high lung deposition efficiency due to small aerodynamic size may also be important in contributing to the health effects (Utell and Frampton 2000). These characteristics result in a higher local dose despite a similar exposure concentration compared to that of micron sized particles.

One major difference between micron and nano sized particles is the different recognition patterns between alveolar macrophages that have an essential role in clearing particles from the lungs, and the epithelial cells, with their function being predominantly facilitation of gas exchange between the air and the blood (oxygen

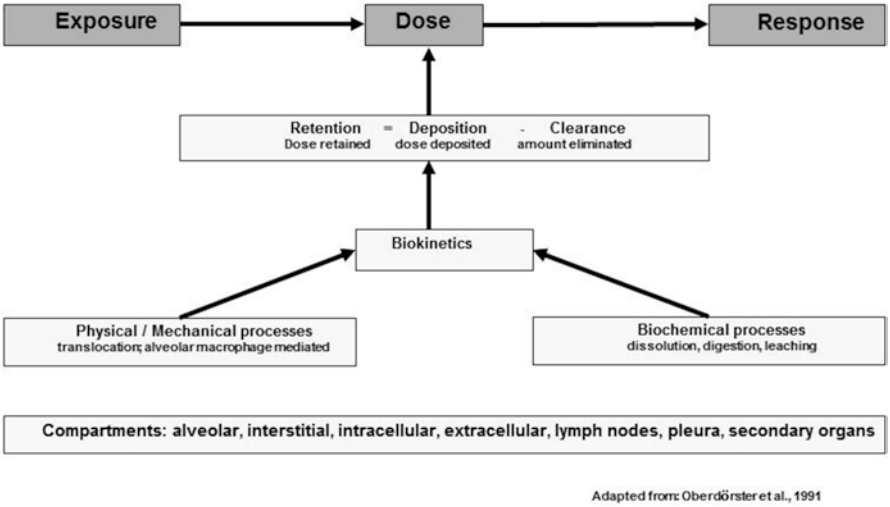


Fig. 1 Biokinetics associated with the exposure–dose–response paradigm

and carbon dioxide). As illustrated in Fig. 2, macrophages may not recognize nano sized particles efficiently which results in a higher dose for the epithelial cells.

Plausible biological mechanisms linking airborne NMs pollution to cardiovascular disease involve (a) direct effects of pollutants on cardiac, endothelial, blood and pulmonary cells and receptors, and/or (b) indirect effects mediated through pollutant-induced pulmonary oxidative stress and inflammatory responses. Direct effects may occur via agents that readily cross the pulmonary epithelium into the circulation, such as gases, and ultrafine particles along with soluble constituents of $PM_{2.5}$ (e.g., transition metals). In addition, activation of the autonomous nervous system secondary to PM interactions with sensory neurons and receptors in the airways may play a role. These direct effects of airborne NMs represent a conceivable explanation for the occurrence of rapid (within a few hours) cardiovascular responses, such as onset of myocardial infarctions in predisposed people. In contrast, less acute (several hours to days) and chronic (days to weeks) indirect effects may occur via pulmonary oxidative stress, inflammation and build-up of morphological changes induced by inhaled pollutants. NMs have been suggested to directly interact with target cells and cross cell barriers. Apart from crossing the air-blood barrier, evidence is emerging that nanoparticles deposit on the olfactory epithelium in the nose and can relocate into the various parts of the brain via the olfactory bulb.

Once NMs are blood born and they will be transported to other parts in the body whereas the liver and the spleen act as efficient filtration systems. However, the latter may also respond with an inflammatory response as can be seen in the lung after exposure to NMs. Thus, the majority of studies into inhalation toxicology suggest that the effect of granular NMs are by and largely driven by the dissolution rate in case of (transition) metals, whereas the adverse responses seen following exposure to fibres are highly dependent on the aspect ratio and sometimes can result in an

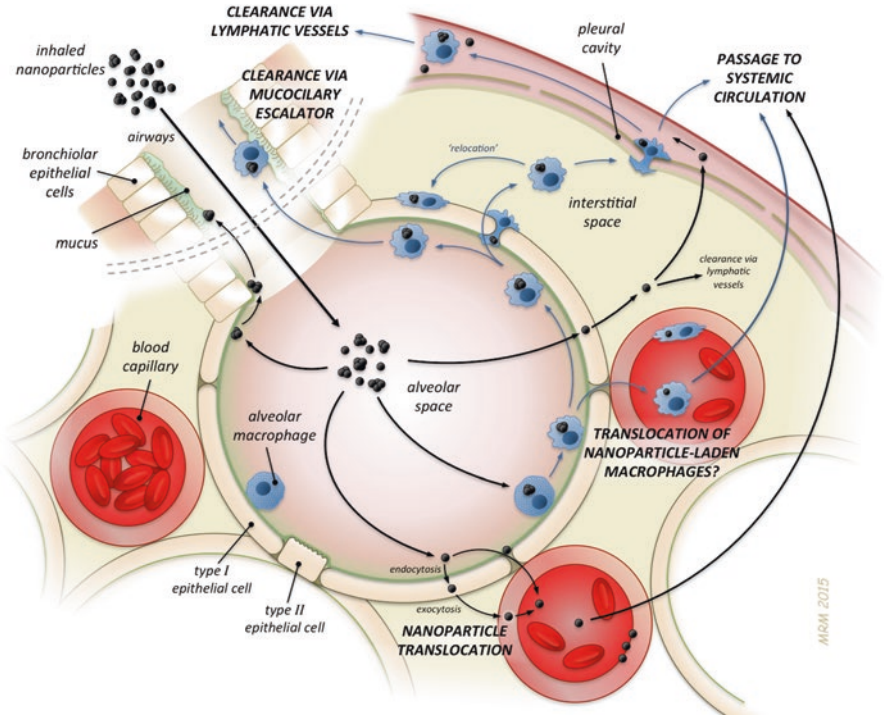


Fig. 2 Schematic presentation of the interaction of particles with the biological (defense) system and translocation mechanism in the lung. Particle can interact or phagocytized by free moving macrophages or can directly interact with the epithelial cells. Cells can response by releasing mediators attracting inflammatory cells (neutrophils). Particles can also pass through cells or in between cells reaching the blood vessels and blood. (Taken from Stone et al. 2017)

asbestos-like effect when materials are rigid and bio-persistent (as discussed above) (Wang et al. 2017; Shvedova et al. 2014).

Based on the literature, it can be considered that titanium dioxide (TiO_2) is the most well-studied NM following inhalation exposure. As with many insoluble particles much of the toxic response is due to the physical aspects rather than a specific chemical-biological reaction. This was clearly demonstrated in studies by Oberdörster and colleagues (Baggs et al. 1997; Oberdörster et al. 2002) who exposed rats and mice by intratracheal instillation of nanoparticles of TiO_2 (20 nm particle diameter) and larger pigment-grade particles (250 nm) establishing dose-response relationships. It was evident that the nanoparticles lead to a greater acute pulmonary inflammatory response as well as fibrosis at a later stage than pigment grade material. Although, on a mass basis, nanoparticles were reported to have a heightened hazard, it was also shown that when using total surface area as the metric for dose, the dose-response curves for both TiO_2 were overlapping for the studies performed in mice and rats (Oberdörster et al. 2000). On the other hand, various studies in which rats were exposed by inhalation (Eydner et al. 2012, Horie et al. 2012)

indicated that very few differences in effects were observed based on differences in particle size. Eydner used a protocol of 6 hours/day, 7 day/week, for 3 weeks up to 45 mg/m^3 for 21 and 300 um particles (Eydner et al. 2012). Subtle if any effects were observed and no clear statistically significant differences between the two types of TiO_2. However, given the fact that in this study the mass median aerodynamic diameter was much more comparable (0.7 vs 1.1 um) than the primary size, more or less similar dose levels will have been obtained in terms of particle numbers and particle mass due to aggregation of primary particles. This most likely explains the relatively small differences that were observed compared to studies done by (Baggs et al. 1997; Oberdörster et al. 2002). Similar results were obtained in a study in which rats were exposed to 0.2 mg nano (7 nm) and fine (200 nm) TiO_2 particles in suspension via intratracheal instillations (Horie et al. 2012). No adverse responses were observed, whereas similar dose levels of nickel oxide caused clear toxicity, up to a week after a single exposure, let alone the significant differences observed that could be related to the size of the particles. The molecular structure of TiO_2 is fixed, yet the physical aspects can be rather variable. Since this also affects the performance of TiO_2, various applications (e.g. photo catalyst, solar energy), and the variability in the physical aspects suggest that the toxicological profile will also change due to differences in dimensions.

Cardiovascular Effects of Pulmonary Exposure

Epidemiological studies have consistently reported a positive relationship between levels of ambient particular matter (PM i.e. particles in air smaller than 10 um aerodynamic diameter) and cardiovascular morbidity (Pope and Dockery 2006, Brook et al. 2010). Causes of increased cardiovascular mortality include: chronic coronary artery disease, congestive heart failure, ischemic heart disease, and cerebrovascular disease. The associations between PM and cardiovascular dysfunction are stronger for $PM_{2.5}$ than PM_{10}, i.e., smaller particles appear to exhibit greater cardiovascular dysfunction on an equal mass basis. It is possible that on an equal mass or surface area basis nanoparticles may be more potent that fine particles. There is increasing, but still limited, epidemiological evidence on the effects of ultrafine (<0.1 um) particles on cardiorespiratory health and the central nervous system. Ultrafines or nanoparticles in air consist mainly out of carbonaceous combustion derived particles with stronger association than those observed for $PM_{2.5}$ and PM_{10} (Janssen et al. 2011; Peters et al. 2011). Several mechanisms, including particle translocation from the lung to the cardiovascular tissue, elevation of inflammatory or thrombotic mediators in blood, oxidant production at systemic vessels, and alteration of automatic control of the cardiovascular system, have been proposed to explain how particle inhalation results in cardiovascular dysfunction (Utell et al. 2002). Albeit that the before mentioned information applies to ambient aerosols containing nano-sized particles it is plausible that similar differences in responses result from exposure to engineered NMs (Vesterdal et al. 2010; Raftis and Miller 2019; Miller et al. 2017).

Three hypothetical pathways to explain the cardiovascular effects of PM predominate; 'inflammation', 'autonomic regulation' and 'particle translocation'. The conventional hypothesis is that particles inhaled into the lung are taken up by alveolar macrophages, eliciting an inflammatory reaction within the lung. An adequate particle dose, reactivity or lack of clearance, leads to augmentation of the response with a resultant 'spill-over' of inflammatory mediators into the blood. This will cause systemic inflammation (Seaton et al. 1995), which is strongly associated with cardiovascular disease. Alternatively, either inhaled particles or the inflammatory response resulting from the particles stimulate alveolar sensory receptors (Ghelfi et al. 2010; Hazari et al. 2011), providing a signal to the central nervous system. This shows from changes in autonomic nervous system activity, which directly regulates cardiac function, and, indirectly, other aspects of the cardiovascular system (Rhoden et al. 2005). The recognition of the UFP fraction of PM paved the way for a third hypothesis: that the minute size of nano-sized particles allows them to relocate by moving through the thin alveolar-capillary wall (by an as-yet undetermined mechanisms) and enter the circulation themselves to directly affect cardiovascular function (Nemmar et al. 2001; Oberdörster et al. 2002).

There is a wealth of evidence for and against each of these theories, but in truth all three are likely to occur, with the contribution of each dependent on the physicochemical properties of the UFP, the cardiovascular endpoint under investigation, and the susceptibility of the person/model being explored (Miller 2014). Furthermore, it is highly likely that many of the subtleties of these pathways have yet to be identified. Reports are rapidly emerging from preclinical models that demonstrate similar cardiovascular effects for NM to that shown for UFP, e.g. altered autonomic function (Harder et al. 2005), impaired vasodilatation (Leblanc et al. 2010; Møller et al. 2011), blood hypercoagulability (Emmerechts et al. 2012), and aggravated atherosclerosis (Mikkelsen et al. 2011). Identification of the biological mechanisms for these parallel observations will have important consequences for both fields of research.

A second proposed mechanism is that pulmonary exposure to NMs causes lung inflammation, which enhances inflammatory or thrombotic mediators in the blood to alter cardiovascular response. Nurkiewicz et al. (2006) reported complete inhibition of the ability of arterioles in the shoulder muscle to respond to dilators at a lung burden of TiO_2 in the rat, which did not cause a significant elevation of inflammatory markers in bronchoalveolar lavage fluid. Similarly, they failed to find any elevations of mRNA for inflammatory mediators in peripheral blood samples. Likewise, Kan et al. (2012) failed to find elevated levels of TNF α or IL-1 either immediately or 24 hours after inhalation of nano TiO_2 (rat lung burden of 10 μg). Li et al. (2010) also failed to observe an increase in plasma levels of inflammatory cytokines and chemokines in mice exposed to SWCNT at pulmonary exposure levels that induced arterial plaque formation. Meng et al. (2012) reported similar results in hypertensive rats after pulmonary exposure to SWCNT, i.e., no changes in blood inflammatory mediators but an elevation in plasma endothelin-1 and angiotensin-1 converting enzyme. Erdely et al. 2011 reported that serum inflammatory markers (IL-6, IL-5, CCL11, CCL22, and CXCL1) were elevated in mice 4 hours after pulmonary

exposure to CNT (40 μg/lung) but returned to normal by 24 hours post-exposure. At this time, an elevation of acute phase proteins (C-reactive protein, haptoglobin, and serum amyloid) was noted. Taken as a whole, currently available data do not strongly support the hypothesis that pulmonary exposure to NMs results in blood mediator levels sufficient to explain the cardiovascular effects reported in the literature.

Nurkiewicz et al. (2006) documented that pulmonary exposure to fine TiO_2 increases adherence of polymorphonuclear leukoctyes (PMN) to microvessel walls in the shoulder muscle of rats 24 hours post-exposure. A later study from this group documented that inhalation of nano TiO_2 (10 μg lung burden in rats) potentiated the generation of reactive oxygen species (ROS) from blood PMN 24 hours post-exposure, and that increased ROS generation was observed at the microvessel wall in the shoulder muscle (Nurkiewicz et al. 2009). They proposed that this ROS generation scavenged dilator-induced nitric oxide produced by endothelial cells and, thus, inhibited microvascular dilation. Indeed, antioxidants reversed the depletion of nitric oxide in arteriolar tissue and the dilator dysfunction seen after inhalation of nano TiO_2. Similar results were reported for coronary arterioles after inhalation of nano TiO_2 (10 μg lung burden) (LeBlanc et al. 2010). The local oxidant stress mechanism for the transduction of pulmonary CNT exposure to cardiovascular effects is supported by data from Li et al. (2007). These results indicate that a significant elevation in heme oxygenase-1 (HO-1) in cardiac and aortic tissue and depression of glutathione in aortic mitochondria were associated with pulmonary aspiration of SWCNT (40 μg/mouse).

Albeit that lung and cardiovascular effects have dominated nanoparticle toxicology research in the last decades, since engineered NMs are increasingly utilized in everyday products and humans are more likely to be exposed to these materials via alternative routes, the most prevalent of these being oral and dermal.

Oral Exposure

Ingestion of NMs can occur directly from food, water or orally administered medicines (Card et al. 2011). In addition, retrograde transfer of NMs by mucocilliary clearance may result in the uptake of materials by the GI tract by subsequent swallowing of materials. It is believed that the vast majority of ingested NMs are rapidly passed through the GI tract and lost via the faeces (Papp et al. 2008; Cho et al. 2013; He et al. 2010), although the harsh environment of the stomach with low pH and enzymatic digestion will change some NMs and their subsequent fate. Also, some NMs will dissolve under GI tract conditions (De Jong et al. 2019). Once again, surface properties of NMs play an important role in their translocation from the GI tract. It has been suggested that charged materials exhibit poor bio-availability due to electrostatic repulsion and mucus entrapment (Hoet et al. 2004). Once in submucosal tissue, NMs are capable of entering the lymphatics and the blood capillaries (Møller et al. 2012).

In a study in which Fischer rats, which were orally, exposed to 14C-labelled C60 fullerenes, it was demonstrated that around 98% of the ingested material were cleared in the faeces within 2 days (Yamago et al. 1995). However, the exposure of F344 rats orally dosed with silver (Ag) NM (56 nm) at 500 mg/kg (high experimental dose) for 90 days resulted in alkaline phosphatase (ALP) and cholesterol changes in the blood indicative of slight liver damage. In addition, histopathologic examination revealed a higher incidence of bile-duct hyperplasia, and/or pigmentation in the treated animals. There was also a dose-dependent accumulation of Ag in all tissues examined. Finally, a significant accumulation of Ag was noted in the liver and the kidneys. In another study Fischer rats were exposed to a single intragastric dose of C_{60} (~1 nm) or single walled carbon nanotubes (SWCNTs) (~2 nm (width) and <1 μm (length) (0.064 and 0.64 mg/kg). The authors showed that both doses of the SWCNTs and C_{60} caused DNA damage in liver (Folkmann et al. 2009). However, the same NMs were not able to induce genotoxicity in colonic mucosa cells. It can be argued that from an evolutionary perspective, enterocytes may be less susceptible to toxic effects of exogenous materials. Another explanation could be related to the age of the rats used in the study (sacrificed at 9 weeks old); it has previously been shown that uptake of 1 μm labelled polystyrene particles was approximately nine times higher in adult (5 months) rats compared to young (6–8 weeks) rats (Seifert et al. 1996). In another study, oral exposure of Sprague-Dawley rats to SiO_2 (~10 nm), Fe_2O_3 (60 nm) and Ag (~10 nm) NMs at doses of up to 2000 mg/kg following a single or repeated exposure (daily for 13 weeks) was carried out. Here, no adverse effects were noted in terms of hematological or histological changes within the GI tract, despite signs of inflammation in hepatic tissues. As another example, exposure of Sprague-Dawley rats to hydrophilic pyrogenic silica NMs (10–25 nm) (537 or 933 mg/kg/day) via the oral route for 84 days resulted in periportal fibrosis in the liver. Further analysis revealed a significantly induced gene expression in a fibrosis-related gene set (van der Zande et al. 2014).

Oral administration is one of the main and most important routes of human NM exposure as well the most widely used method of delivering drugs (Kermanizadeh et al. 2018). The stability/bio-availability of NMs in the GIT is complex due to variability of pH in the biological environment, a protective mucus layer and presence of digestive enzymes. This issue is further complicated by the fact that the different physiochemical surface characteristics of different NMs will influence their cellular interactions and uptake and might include absorption, paracellular pathway or transcellular mechanism via clathrin and caveolae-dependent endocytosis (Ma et al. 2014). After oral administration, the common absorption site for NMs is the small intestine. The major cell types in the small intestine are absorptive enterocytes, mucus secretory goblet cells and the immune sampling M cells of the Peyer's patches (Kraehenbuhl and Neutra 2000; Ma et al. 2014). The M cells are associated with lymphocytes, immunoblasts, plasma cells and macrophages and could be important in the initiation of immune responses to NM exposure. This is similar to antigen delivery to dendritic cells or lymphocytes via transcytosis, as well as the transport through epithelial cells via endocytosis, persorption through gaps from shredding at villous tips and ineffective tight junctions (Møller et al. 2012). From

the available data, it is apparent that NM adsorption in the GI tract decreases with increasing material size. Therefore, NM agglomeration influences bio-availability of the original NMs. In the GI tract, the intraluminal pH changes rapidly from being highly acidic in the stomach to about pH 6 in the duodenum from where it gradually increases to about pH 7.4 in the terminal ileum (Marasini et al. 2014). Both homogeneous agglomeration (NM-NM) and heterogeneous agglomeration (NM with dissolved organics – "protein corona") contribute to increased diameters.

As a consideration, it is important to note that uptake can differ between species as demonstrated by a generalized ranking of rabbit > rat > hamster > mice (Delie 1998; Florence 2005). One of the most popular theories proposed for these differences is that animals with higher number of Peyer's patches such as rabbit will have a higher rate of uptake than others. Additionally, it has been suggested that the rate of uptake may also be subject to change with older animals capable of much higher uptake compared to the younger animals (better functionality of Peyer's patches with age). Finally, the immunological state of the animal might be pivotal as it has been demonstrated that the Peyer's patch numbers increase when the mucosal system is triggered (Becker et al. 2012).

Oral NM application by gavage (instead of administration in the food or drinking water) has been recommended as being suitable to ensure well-defined conditions of the test substance administration, since it allows delivering a precise dose of the NM to the animals with a well-characterized degree of dispersion (Hadrup and Lam 2014). However, it is important to remember that NM application by gavage does not reflect the lower test substance concentrations delivered over longer periods of time when NMs are administered with the animals' feed. Oral gavage utilizes a bolus of NMs that may or may not mix with the gastrointestinal fluids, thereby possibly resulting in a higher local NM concentration and hence increased quantity of the absorbed material. In the preponderance of the investigations, effects were only observed by histopathological examination or clinical chemistry, without any corresponding clinical findings. Therefore, it is difficult, if not impossible, to determine the relevance such adverse effects, and it is not yet possible to come to a conclusion in regard to, i.e. ranking of toxic effects of different types of NMs upon oral exposure. Nevertheless, the assessment of uptake across a range of NMs indicates that the translocation from the GI tract to distal organs is rather small – typically less than 1% of the administered dose. The majority of the studies have observed NMs or constituents thereof (e.g. metals) in the liver and the spleen.

In general, in vivo studies comparing the effects of different NMs under identical experimental conditions or assessing organ burden upon oral NM administration are very rare. Often, experiments were not conducted in accordance with standardized test guidelines, which makes it difficult to generalize between investigations (Kermanizadeh et al. 2015). Furthermore, comparative hazard assessments of different NMs under identical experimental conditions are lacking, as are investigations on the reversibility or progression of effects (Kermanizadeh et al. 2015). Therefore, it is difficult, if not impossible, to determine the relevance such adverse effects, and it is not yet possible to conclude, or even rank the toxicity of different types of NMs following oral exposure.

Dermal Exposure

The human skin is composed of three layers being the subcutaneous tissue, the dermis and epidermis. The superficial epidermis provides a protective barrier against foreign invaders, regulates release of water from the body and protects the body from harm due to exposure to e.g. chemicals, NMs. The epidermis consists of keratinocytes that show a differentiation from living and dividing basal keratinocytes into the non-living superficial stratum corneum, the keratin layer covering the skin. NMs (NMs) have been used for the last decennia to protect the skin from exposure to UV sunlight notably by the use of ZnO- and TiO_2 nanoparticles as inorganic UV filters in sunscreens (TGA 2016). Both NMs are considered safe for their use in sunscreens although for TiO_2-NPs for some products limitations exist in view of their potential for photocatalytic activity and possibility to be phototoxic (SCCS 2012, 2014a, b; TGA 2016).

Regarding the potential hazards for exposure of the skin to NMs two aspects needs to be considered one being the "local" toxicity in terms of potential for irritation and sensitization, and one being the potential for penetration of the skin and resulting internal systemic exposure. In addition, for local toxicity also potential genotoxicity/mutagenicity should be considered. Dermal exposure can occur due to accidental spillage or low hygienic occupational settings or due to purpose made consumer products for skin application such as sunscreens. The sensitization potential of NMs is relatively unknown, one reason being the difficulty in how testing for sensitizing potency of NMs should be performed. The use of intradermal injection at the base of the ear in mice is described as an alternative for the local lymph node assay (LLNA) that normally uses topical skin application on the outer ear (Hussain et al. 2012). Injection of TiO_2 NPs in acetone-olive-oil (AOO)-treated control mice did not have any effect on lymph node (LN) proliferation as indication for immune stimulation. Dinitrochlorobenzene (DNCB) sensitization resulted in LN proliferation, which was further increased by injection of TiO_2 NPs before DNCB sensitization. In a follow-up study topical exposure of TiO_2, Ag or SiO_2 nanoparticles did not induce an immune response in the draining local lymph node when applied on the skin. However, some local activity did occur as treatment with the allergen DNCB after the topical NMs administration, enhanced the DNCB response (Smulders et al. 2015). In both studies no immune stimulation in the draining lymph node was noted as indication for sensitization potential, however, in combination with the well-known model sensitizer DNCB enhancement of the DNCB response was observed indicating adjuvant activity for the TiO_2, Ag and SiO_2 NPs.

Several studies showed that NPs (e.g. ZnO, Ag, TiO_2, and CeO_2 nanoparticles) do not show local irritation activity after evaluation in a reconstructed human epidermis (RhE) model (Vinardell and Mitjans 2017; Miyani and Hughes 2017). In this model the NMs can be applied in both a watery and lipid solution on top of the epidermal construct that has a similar tissue layers as normal human skin. Six RhE models were validated and accepted for determination of irritant activity of chemicals in OECD TG 439 (OECD 2019). Also, in *in vivo* tests skin irritation was not

observed after 14-day topical administration of coated and non-coated silver NMs to the skin of weanling pigs (Monteiro-Riviere 2016). Silver nanoparticles were only observed in the upper layers of the epidermis (Samberg et al. 2010). Local toxicity in the skin was absent after repeated application of C_{60} fullerenes (Xia et al. 2010).

Skin penetration is poorly understood as many factors have not been properly studied yet (Monteiro-Riviere and Larese Filon 2017). In particular, long-term skin exposure studies are lacking. Local penetration of NMs in the skin is mainly limited to the first superficial layers of the stratum corneum (Butz et al. 2007; Samberg et al. 2010). Local accumulation in hair follicles may occur (Chaudhry 2015). However, after dermal exposure to ZnO NPs (20 nm), ^{68}Zn originating from the dermally applied NMs in sunscreens could be detected in the blood of treated volunteers (Gulson et al. 2010). With regard to skin penetration and a potential risk for systemic exposure, it should be realized that the presence of skin damage can affect the skin penetration of NMs. So, the quality of the skin (damage like abrasions, sunburns) but also stretching and the presence of solvents in the product/nanodispersion can influence dermal penetration and uptake (Monteiro-Riviere and Larese Filon 2012; Holmes et al. 2016). For Ag NPs it was noted that there was no penetration into viable skin whereas in damaged (burnt skin) Ag NPs were able to reach the viable cells in the dermis (Holmes et al. 2016). On the other hand specifically formulated and coated NMs are now evaluated to increase skin penetration for medical applications (Lin et al. 2018). In the RhE model it was demonstrated that the skin penetration of Au nanoparticles was dependent on the coating with the cetyltrimethylammonium bromide-coated (CTAB) coated gold nanoparticles with positive surface charges exhibiting the highest efficiency in skin penetration (Hao et al. 2017). The skin penetration was due to compromised tight junctions of keratinocytes, causing paracellular penetration of NPs.

When we consider various aspects of the possibility of NMs to penetrate the skin the physicochemical characteristics play an important role (Larese Filon et al. 2015). A difference should be made between metal and non-metal NPs. Size dependent penetration seems possible with NPs $\leq$4 nm able to penetrate intact skin, NPs with sizes between 4 and 20 nm can penetrate intact and damaged skin, and NPs with sizes between 21 and 45 nm can only damaged skin (as reviewed by Larese Filon et al. 2015). For metal NPS also the solubility plays an important role that can result in local and/or systemic (allergic) effects. So, for skin toxicity three aspects need to be considered being size in physiological media, chemical composition with regard to possible intrinsic toxicity, and solubility regarding release of toxic metals.

Although skin penetration for solid nanoparticles such as metals and metal oxides is limited, the use of dedicated coatings and NM formulations (e.g. soft NMs like liposomes) can enhance penetration and uptake of NMs by the skin. The condition of the skin itself, e.g. localized damage like abrasions or sunburn, may also increase the uptake of NMs via the skin. For partially soluble NMs (ZnO nanoparticles) systemic availability of the metal ions could be demonstrated.

Conclusions

Almost three decades of intense research into the toxicology of engineered NMs has revealed limited understanding as to the direct impact upon human health. As with most cases, there will be exceptions as has been showed for more fibre shaped materials such certain carbon nanotubes, as these can potentially lead to the same type of effects that are caused by asbestos, including mesothelioma, if they exhibit the precise same characteristics as these pathogenic fibres. It is also clear that the kinetics of NMs is different from micro-sized materials, with a higher likelihood (though also at a very low rate) to reach secondary organs. Very little information is available to draw firm conclusions on the implications of long-term low dose exposure and as to what extend this will lead to accumulation of NMs in the body. From acute exposures studies it can be concluded that the clearance rate of NMs once taken up in the body is lower than from larger sized materials. As engineered NMs are purposely made, it also opens opportunities from minimizing the toxicity already in the design phase, by avoiding those properties that potentially make materials harmful. This often referred to as safe-by-design and would be beneficial from both the manufacturers (reduction of cost, avoiding bringing harmful products to the market) and those that are exposed to the materials (reducing health risks).

References

Baggs RB, Ferin J, Oberdörster G. Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. Vet Pathol. 1997;34(6):592–7.

Becker HM, Bertschinger MM, Rogler G. Microparticles and their impact on intestinal immunity. Dig Dis. 2012;30:47–54.

Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC, Whitsel L, Kaufman JD. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American heart association. Circulation. 2010;121:2331–78.

Butz T, Reinert T, Pinheiro T, Moretto P, Pallon J, Kiss AZ, Stachura J, D¸Abro's W, Stachura Z, Lekki J, Lekka M, Hunyadi J, Bıro T, Sticherling M, Van Vaeck L, Van Royen P, Surleve-Bazeille JE. NANODERM, Quality of skin as a barrier to ultra-fine particles, QLK4-CT-2002-02678 final report. 2007.

Card JW, Jonaitis TS, Tafazoli S, Magnuson BA. An appraisal of the published literature on the safety and toxicity of food-related NMs. Crit Rev Toxicol. 2011;41:22–49.

Chaudhry Q. Opinion of the Scientific Committee on Consumer safety (SCCS) – revision of the opinion on the safety of the use of titanium dioxide, nano form, in cosmetic products. Regul Toxicol Pharmacol. 2015;73:669–70.

Cho WS, Kang BC, Lee JK, Jeong J, Che JH, Seok SH. Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Part Fibre Toxicol. 2013;10:9.

De Jong WH, De Rijk E, Bonetto A, Wohlleben W, Stone V, Brunelli A, Badetti E, Marcomini A, Gosens I, Cassee FR. Toxicity of copper oxide and basic copper carbonate nanoparticles after short-term oral exposure in rats. Nanotoxicology. 2019;13:50–72.

Delie F. Evaluation of nano- and microparticle uptake by the gastrointestinal tract. Adv Drug Deliv Rev. 1998;34:221–33.

Erdely A, Liston A, Salmen-Muniz R, Hulderman T, Young SH, Zeidler-Erdely PC, Castranova V, Simeonova PP. Identification of systemic markers from a pulmonary carbon nanotube exposure. J Occup Environ Med. 2011;53(6 Suppl):S80-6.

Emmerechts J, Jacobs L, Van Kerckhoven S, Loyen S, Mathieu C, Fierens F, Nemery B, Nawrot TS, Hoylaerts MF. Air pollution-associated procoagulant changes: the role of circulating microvesicles. J Thromb Haemost. 2012;10(1):96–106. https://doi.org/10.1111/j.1538-7836.2011.04557.x.

Eydner M, Schaudien D, Creutzenberg O, Ernst H, Hansen T, Baumgärtner W, Rittinghausen S. Impacts after inhalation of nano- and fine-sized titanium dioxide particles: morphological changes, translocation within the rat lung, and evaluation of particle deposition using the relative deposition index. Inhal Toxicol. 2012;24(9):557–69.

Florence AT. Nanoparticle uptake by the oral route: fulfilling its potential? Drug Discov Today Technol. 2005;2:75–81.

Folkmann JK, Risom L, Jacobsen NR, Wallin H, Loft S, Møller P. Oxidatively damaged DNA in rats exposed by oral gavage to C60 fullerenes and single-walled carbon nanotubes. Environ Health Perspect. 2009;117:703–8.

Geiser M, Kreyling WG. Deposition and biokinetics of inhaled nanoparticles. Part Fibre Toxicol. 2017;7:2.

Ghelfi E, Wellenius GA, Lawrence J, Millet E, Gonzalez-Flecha B. Cardiac oxidative stress and dysfunction by fine concentrated ambient particles (caps) are mediated by angiotensin-ii. Inhal Toxicol. 2010;22:963–72.

Gulson B, Mccall M, Korsch M, Gomez L, Casey P, Oytam Y, Taylor A, Mcculloch M, Trotter J, Kinsley L, Greenoak G. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci. 2010;118:140–9.

Gulumian M, Verbeek J, Andraos C, Sanabria N, de Jager P. Systematic review of screening and surveillance programs to protect workers from NMs. PLoS One. 2016;11(11):e0166071.

Hadrup N, Lam HR. Oral toxicity of silver ions, silver nanoparticles and colloidal silver – a review. Regul Toxicol Pharmacol. 2014;68:1–7.

Han JH, Lee EJ, Lee JH, So KP, Lee YH, Bae SN, Lee SB, Ji JH, Cho MH, Yu IJ. Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility. Inhal Toxicol. 2008;20:741–9.

Hao F, Jin X, Liu QS, Zhou Q, Jiang G. Epidermal penetration of gold nanoparticles and its underlying mechanism based on human reconstructed 3D Episkin model. ACS Appl Mater Interfaces. 2017;9:42577–88.

Harder V, Gilmour PS, Lentner B, Karg E, Takenaka S, Ziesenis A, et al. Cardiovascular responses in unrestrained WKY rats to inhaled ultrafine carbon particles. Inhal Toxicol. 2005;17:29–42.

Hazari MS, Haykal-Coates N, Winsett DW, Krantz QT, King C, Costa DL, et al. Trpa1 and sympathetic activation contribute to increased risk of triggered cardiac arrhythmias in hypertensive rats exposed to diesel exhaust. Environ Health Perspect. 2011;119:951–7.

He X, Zhang H, Ma Y, Bai W, Zhang Z, Lu K, et al. Lung deposition and extrapulmonary translocation of nano-ceria after intratracheal instillation. Nanotechnology. 2010;21:285103.

Hoet PHM, Hohlfeld IB, Salata O. Nanoparticles – known and unknown health risks. J Nanobiotechnol. 2004;2:12–27.

Holmes AM, Lim J, Studier H, Roberts MS. Varying the morphology of silver nanoparticles results in differential toxicity against micro-organisms, HaCaT keratinocytes and affects skin deposition. Nanotoxicology. 2016;10:1503–14.

Horie M, Fukui H, Endoh S, Maru J, Miyauchi A, Shichiri M, Fujita K, Niki E, Hagihara Y, Yoshida Y, Morimoto Y, Iwahashi H. Comparison of acute oxidative stress on rat lung induced by nano and fine-scale, soluble and insoluble metal oxide particles: NiO and TiO2. Inhal Toxicol. 2012;24(7):391–400.

Hussain S, Smulders S, De Vooght V, Ectors B, Boland S, Marano F, Van Landuyt KL, Nemery B, Hoet PH, Vanoirbeek JA. Nano-titanium dioxide modulates the dermal sensitization potency of DNCB. Part Fibre Toxicol. 2012;9:15.

Janssen NA, Hoek G, Simic-Lawson M, Fischer P, van Bree L, ten Brink H, Keuken M, Atkinson RW, Anderson HR, Brunekreef B, Cassee FR. Black carbon as an additional indicator of the adverse health effects of airborne particles compared with PM10 and PM2.5. Environ Health Perspect. 2011;119(12):1691–9.

Johnston CJ, Finkelstein JN, Mercer P, Corson N, Gelein R, Oberdörster G. Pulmonary effects induced by ultrafine PTFE particles. Toxicol Appl Pharmacol. 2000;168(3):208–15.

Kan H, Wu ZX, Young SH, Chen BT, Cumpston JL, Chen F, Kashon ML, Castranova V. Pulmonary exposure of rates to ultrafine titanium dioxide enhances cardiac protein phosphorylation and substance P synthesis in nodose ganglia. Nanotoxicol. 2012;6:736–45.

Kermanizadeh A, Balharry D, Wallin H, Loft S, Møller P. NM translocation – the biokinetics, tissue accumulation, toxicity and fate of materials in secondary organs – a review. Crit Rev Toxicol. 2015;45:837–72.

Kermanizadeh A, Powell LG, Stone V, Møller P. Nano delivery systems and stabilized solid drug nanoparticles for orally administered medicine – current landscape. Int J Nanomedicine. 2018;13:7575–605.

Kraehenbuhl JP, Neutra MR. Epithelial M cells: differentiation and function. Annu Rev Cell Dev Biol. 2000;16:301–32.

Kreyling WG, Semmler-Behnke M, Takenaka S, Moller W. Differences in the biokinetics of inhaled nano – versus micrometer-sized particles. Acc Chem Res. 2013;46:714–22.

Kuempel ED, Sweeney LM, Morris JB, Jarabek AM. Advances in inhalation dosimetry models and methods for occupational risk assessment and exposure limit derivation. J Occup Environ Hyg. 2015;12(sup1):S18–40.

Larese Filon F, Mauro M, Adami G, Bovenzi M, Crosera M. Nanoparticles skin absorption: new aspects for a safety profile evaluation. Regul Toxicol Pharmacol. 2015;72:310–22.

LeBlanc AJ, Moseley AM, Chen BT, Frazer D, Castranova V, Nurkiewicz TR. Nanoparticles inhalation impairs coronary microvascular reactivity via a local reactive oxygen-dependent mechanism. Cardiovasc Toxicol. 2010;10:27–36.

Lee JH, Lee SB, Bae GN, Jeon KS, Yoan JU, Ji JH, Sung JH, Lee BG, Lee JH, Yang JS, Kim HY, Kung CS, Yu SJ. Exposure assessment of carbon nanotube manufacturing workplaces. Inhal Toxicol. 2010;22:369–81.

Lee JS, Choi YC, Shin JH, Lee JH, Lee Y, Park SY, et al. Health surveillance study of workers who manufacture multi-walled carbon nanotubes. Nanotoxicology. 2015;9:802–11.

Li Z, Hulderman T, Salmen R, Chapmen R, Leonard SS, Young S-H, Shvedova A, Luster MI, Simeonova P. Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environ Health Perspect. 2007;115:377–82.

Li M, Al-Jamal KT, Kostarelos K, Reinke J. Physiologically based pharmacokinetic modeling of nanoparticles. ACS Nano. 2010;4:6303–017.

Liao H-Y, Chung Y-T, Lai C-H, Wang S-L, Chiang H-C, Li L-A, et al. Six-month follow-up study of health markers of NMs among workers handling engineered NMs. Nanotoxicology. 2014;8:100–10.

Lin H, Xie Q, Huang X, Ban J, Wang B, Wei X, Chen Y, Lu Z. Increased skin permeation efficiency of imperatorin via charged ultradeformable lipid vesicles for transdermal delivery. Int J Nanomedicine. 2018;13:831–42.

Ma T, Wang L, Yang T, Ma G, Wang S. M-cell targeted polymeric lipid nanoparticles containing a toll-like receptor agonist to boost oral immunity. Int J Pharm. 2014;473:296–303.

Marasini N, Skwarczynski M, Toth I. Oral delivery of nanoparticle-based vaccines. Expert Rev Vaccines. 2014;13:1361–76.

Maynard AD, Baron PA, Foley M, Shvedova AD, Kisin ER, Castranova V. Exposure to carbon nanotube material: aerosol release during the handling of unrefined single walled carbon nanotube material, J. Toxicol. Environ. Health Part A. 2004;67:87–107.

Meng GC, Xu L, Bai R, Du J, Zhang L, Li Y, Chang Y, Zhao Y, Chen C. Acute pulmonary and moderate cardiovascular responses of spontaneously hypertensive rats after exposure to single-wall carbon nanotubes. Nanotoxicology. 2012;6:526–42.

Methner MM. Effectiveness of local exhaust ventilation (LEV) in controlling engineered NM emissions during reactor cleanout operations. J Occup Environ Hyg. 2008;5:D63–9.

Mikkelsen L, Sheykhzade M, Jensen KA, Saber AT, Jacobsen NR, Vogel U, et al. Modest effect on plaque progression and vasodilatory function in atherosclerosis-prone mice exposed to nano-sized TiO 2. Part Fibre Toxicol. 2011;8:32.

Miller MR. The role of oxidative stress in the cardiovascular actions of particulate air pollution. Biochem Soc Trans. 2014;42(4):1006–11. https://doi.org/10.1042/BST20140090.

Miller MR, Raftis JB, Langrish JP, McLean SG, Samutrtai P, Connell SP, Wilson S, Vesey AT, Fokkens PHB, Boere AJF, Krystek P, Campbell CJ, Hadoke PWF, Donaldson K, Cassee FR, Newby DE, Duffin R, Mills NL. Inhaled nanoparticles accumulate at sites of vascular disease. ACS Nano. 2017;11(5):4542–52.

Miyani VA, Hughes MF. Assessment of the in vitro dermal irritation potential of cerium, silver, and titanium nanoparticles in a human skin equivalent model. Cutan Ocul Toxicol. 2017;36:145–51.

Møller P, Mikkelsen L, Vesterdal LK, Folkmann JK, Forchhammer L, Roursgaard M, et al. Hazard identification of particulate matter on vasomotor dysfunction and progression of atherosclerosis. Crit Rev Toxicol. 2011;41:339–68.

Møller P, Folkmann JK, Danielsen PH, Jantzen K, Loft S. Oxidative stress generated damage to DNA by gastrointestinal exposure to insoluble particles. Curr Mol Med. 2012;12:732–45.

Monteiro-Riviere NA. Safety of nanoparticle skin penetration. In: Dragicevic N, Maibach HI, editors. Percutaneous penetration enhancers—chemical methods in penetration enhancement: nanocarriers. Berlin/Heidelberg: Springer; 2016.

Monteiro-Riviere NA, Larese Filon F. Chapter 11 – Skin. In: Fadeel B, Pietroiusti A, Shvedova A, editors. Adverse effects of engineered NMs, 1st edition. Exposure, toxicology, and impact on human health. New York: Elsevier; 2012. p. 185–207.

Monteiro-Riviere NA, Larese Filon F. Chapter 15 – Effects of engineered NMs on skin. In: Fadeel B, Pietroiusti A, Shvedova A, editors. Adverse effects of engineered NMs. Exposure, toxicology, and impact on human health. 2nd ed. London: Elsevier/Academic Press; 2017. p. 357–80.

Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PH, Verbruggen A, Nemery B. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am J Respir Crit Care Med. 2001;164:1665–8.

Nurkiewicz TR, Porter DW, Barger M, Millecchia L, Rao KM, Marvar PJ, Hubbs AF, Castranova V, Boegehold MA. Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. Environ Health Perspect. 2006;114:412–9.

Nurkiewicz TR, Porter DW, Hubbs AF, Stone S, Chen BT, Frazer DG, Boegehold MA, Castranova V. Pulmonary nanoparticle exposure disrupts systemic microvascular nitric oxide signaling. Toxicol Sci. 2009;110:191–203.

Oberdörster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R, Elder AC. Acute pulmonary effects of ultrafine particles in rats and mice. Res Rep Health Eff Inst. 2000;(96):5–74; disc. 75–86.

Oberdörster G, Sharp Z, Atudoriei V, Eleder A, Gelein R, Lunts A, Kreyling W, Cox C. Extrapulmonary translocation of ultrafine carbon particles following whole body inhalation exposure of rats, J. Toxicol. Environ. Health Part A. 2002;65:1531–43.

OECD. Guidelines for the testing of chemicals. Test no. 439: in vitro skin irritation: reconstructed human epidermis test method. Paris: OECD; 2019.

Papp T, Schiffmann D, Weiss D, Castronova V, Vallyathan V, Rahman Q. Human health implications of NM exposure. Nanotoxicology. 2008;2:9–27.

Peters A, Rückerl R, Cyrys J. Lessons from air pollution epidemiology for studies of engineered NMs. J Occup Environ Med. 2011;53(6 Suppl):S8–S13.

Pope CA 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc. 2006;56:709–42.

Raftis JB, Miller MR. Nanoparticle translocation and multi-organ toxicity: a particularly small problem. Nano Today. 2019;26:8–12.

Rhoden CR, Wellenius GA, Ghelfi E, Lawrence J, González-Flecha B. PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. Biochim Biophys Acta. 2005;1725(3):305–13. Epub 2005 Jun 20

Riebeling C, Luch A, Götz ME. Comparative modeling of exposure to airborne nanoparticles released by consumer spray products. Nanotoxicology. 2016;10(3):343–51.

Riediker M, Zink D, Kreyling W, Oberdörster G, Elder A, Graham U, Lynch I, Duschl A, Ichihara G, Ichihara S, Kobayashi T, Hisanaga N, Umezawa M, Cheng T-J, Handy R, Gulumian M, Tinkle S, Cassee F. Particle toxicology and health – where are we? Part Fibre Toxicol. 2019;16:19.

Samberg ME, Oldenburg SJ, Monteiro-Riviere NA. Evaluation of silver nanoparticle toxicity in skin in vivo and keratinocytes in vitro. Environ Health Perspect. 2010;118(3):407–13.

SCCS (Scientific Committee on Consumer Safety). Opinion on ZnO (nano form), 18 September 2012. European Commission, Brussels, Belgium. 2012.

SCCS (Scientific Committee on Consumer Safety). ADDENDUM to the Opinion SCCS/1489/12 on Zinc oxide (nano form), 23 July 2013, revision of 22 April 2014. European Commission, Brussels, Belgium. 2014a.

SCCS (Scientific Committee on Consumer Safety). Opinion on titanium dioxide (nano form), 22 July 2013, revision of 22 April 2014. European Commission, Brussels, Belgium. 2014b.

Seaton A, MacNee W, Donaldson K, Godden D. Particulate air pollution and acute health effects. Lancet. 1995;345(8943):176–8.

Seifert J, Haraszti B, Sass W. The influence of age and particle number on absorption of polystyrene particles from the rat gut. J Anat. 1996;189:483–6.

Shvedova AA, Yanamala N, Kisin ER, Tkach AV, Murray AR, Hubbs A, Chirila MM, Keohavong P, Sycheva LP, Kagan VE, Castranova V. Long-term effects of carbon containing engineered NMs and asbestos in the lung: one year postexposure comparisons. Am J Physiol Lung Cell Mol Physiol. 2014;306(2):L170–82.

Shvedova AA, Yanamala N, Kisin ER, Khailullin TO, Birch ME, Fatkhutdinova LM. Integrated analysis of dysregulated ncRNA and mRNA expression profiles in humans exposed to carbon nanotubes. PLoS One. 2016;11:e0150628.

Smulders S, Golanski L, Smolders E, Vanoirbeek J, Hoet PH. Nano-TiO2 modulates the dermal sensitization potency of dinitrochlorobenzene after topical exposure. Br J Dermatol. 2015;172:392–9.

Stone V, Miller MR, Clift MJD, Elder A, Mills NL, Møller P, Schins RPF, Vogel U, Kreyling WG, Alstrup Jensen K, Kuhlbusch TAJ, Schwarze PE, Hoet P, Pietroiusti A, De Vizcaya-Ruiz A, Baeza-Squiban A, Teixeira JP, Tran CL, Cassee FR. NMs versus ambient ultrafine particles: an opportunity to exchange toxicology knowledge. Environ Health Perspect. 2017;125(10):106002.

TGA (Therapeutic Goods Administration). Literature review on the safety of titanium dioxide and zinc dioxide nanoparticles in sunscreens. Scientific Review Report. TGA, Department of Health, Australian Government. 2016. https://www.tga.gov.au/sites/default/files/nanoparticles-sunscreens-review-_2016_1.pdf

Utell MJ, Frampton MW. Acute health effects of ambient air pollution: the ultrafine particle hypothesis. J Aerosol Med. 2000;13(4):355–9.

Utell MJ, Frampton MW, Zareba W, Devlin RB, Cascio WE. Cardiovascular effects associated with air pollution: potential mechanisms and methods of testing. Inhal Toxicol. 2002;14:1231–47.

van der Zande M, Vandebriel RJ, Groot MJ, Kramer E, Rivera ZEH, Rasmussen K, Ossenkoppele JS, Tromp P, Gremmer ER, Peters RJB, Henderiksen PJ, Marvin HJP, Hoogenboom RLAP, Peijnenburg ACAM, Bouwmeester H. Sub-chronic toxicity study in rats orally exposed to nanostructured silica. Part Fibre Toxicol. 2014;11:8.

Vesterdal LK, Folkmann JK, Jacobsen NR, Sheykhzade M, Wallin H, Loft S, Møller P. Pulmonary exposure to carbon black nanoparticles and vascular effects. Part Fibre Toxicol. 2010;7:33.

Vinardell MP, Mitjans M. Alternative methods to animal testing for the safety evaluation of cosmetic ingredients: an overview. Cosmetics. 2017;4:30. 14 pp

Wang J, Schlagenhauf L, Setyan A. Transformation of the released asbestos, carbon fibers and carbon nanotubes from composite materials and the changes of their potential health impacts. J Nanobiotechnol. 2017;15:15.

Willhite CC, Karyakina NA, Yokel RA, Yenugadhati N, Wisniewski TM, Arnold IMF, Momoli F, Krewski D. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. Crit Rev Toxicol. 2016;44(Suppl 4):1–80.

Xia XR, Monteiro-Riviere NA, Riviere JE. Skin penetration and kinetics of pristine fullerenes (C60) topically dosed in industrial organic solvents. Toxicol Appl Pharmacol. 2010;242:29–37.

Yamago S, Tokuyama H, Nakamura E, Kikuchi K, Kananishi S, Sueki K, Nakahara H, Enomoto S, Ambe F. In vivo biological behavior of a water-miscible fullerene: 14C labeling, absorption, distribution, excretion and acute toxicity. Chem Biol. 1995;2(6):385–9.

Nanotoxicology in the Environment

Yueyang Zhang and Greg Goss

Contents

Abstract The recent rapid growth of the nanotechnology industry and the development of new nanomaterials have provided various benefits due to their high efficiency and effectiveness while raising both new threats to the environment and challenges to toxicologists and regulators. While the characteristics of nanomaterial toxicity in model systems have been relatively well studied, the impact of environmental factors on the toxicity of nanomaterials in the environment is still in its infancy. The complexity of the interaction between various environmental factors (ionic strength environmental pH, natural organic matter and ultraviolet light) and nanomaterials are described herein with a call for a comprehensive characterization of nanomaterials in natural environments and performance of experiments under more ecologically relevant conditions and concentrations.

Keywords Nanomaterials · Nanotoxicology · Ecotoxicity · UV · NOM

Y. Zhang
Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

G. Goss (✉)
Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

National Institute for Nanotechnology, Edmonton, AB, Canada

Office of Environmental Nanosafety, University of Alberta, Edmonton, AB, Canada
e-mail: greg.goss@ualberta.ca

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_3

Introduction

Nanomaterials (NMs) have been present in the environment since before the existence of humans (Klaine et al. 2008; Grillo et al. 2015). The natural events on Earth, including volcanic eruptions, forest fires, and dust storms, have been contributing non-engineered NMs to the environment for many years (Klaine et al. 2008; Grillo et al. 2015). These NMs include particulate materials in the atmosphere, colloid and natural organic matter (NOM) in aquatic systems, and minerals and NOM in the soils (Klaine et al. 2008). On the other hand, engineered NMs are intentionally designed and manufactured for specific purposes (Grillo et al. 2015). The modern field of nanoscience and nanotechnology has been rapidly advancing, and the industry has been proliferating since the first introduction of the concept by the Nobel Laureate and quantum theorist, Richard Feynman, in 1959 (Feynman 2012). Nanotechnology is now a multi-billion dollar industry and has been growing rapidly every year (Schultz et al. 2014; Inshakova and Inshakov 2017). The worldwide market of nanotechnology-based products was estimated to be approximately 15 billion United States dollars (USD) (Inshakova and Inshakov 2017) in 2015 (the latest year with publically available data) and the field is expected to have more than 15% annual growth in the next 5 years (He et al. 2018). According to StatNano, more than 8000 nanotechnology-based products are commercially available worldwide in 2018 (StatNano 2018). These include not only high-end applications, including quantum dots in imaging and nano-enabled medical products (Schultz et al. 2014), but also daily consumer goods, such as titanium dioxide (TiO_2) nanoparticles (NPs) in sunscreen, cerium oxide (CeO_2) NPs as diesel fuel additives and nano-enabled pesticides (Bystrzejewska-Piotrowska et al. 2009). Although the use of NMs provides many benefits in various applications, much of these NMs will undoubtedly end up in the environment either through the direct application (e.g., nano-enabled pesticides), via direct release from wastewater treatment plants or indirect release due to the end life cycle of various nanotechnology-based products (Mitrano et al. 2015). In each case, the NM (pristine or transformed during the life cycle), will interact with numerous environmental factors and organisms (Zhang et al. 2009; Keller et al. 2010). As such, it is evident that it is necessary to evaluate the environmental risk of NMs in ecologically relevant conditions to accurately assess risk (Grillo et al. 2015; Zhang et al. 2009; Keller et al. 2010).

NMs are materials with a length of 1–100 nm in at least one dimension and often have unique properties compared to their bulk and dissolved form counterparts (Schultz et al. 2014). The specific surface area (SSA) of NMs increases exponentially with decreasing diameter, which can act to significantly increase the ability to move through the environment, interact with environmental factors, affect cellular uptake rate, and move between compartments inside an animal (Schultz et al. 2014; Bystrzejewska-Piotrowska et al. 2009). As size decreases, there is a significantly increased potential of NMs to interact with the surrounding environment and living organisms (Schultz et al. 2014; Bystrzejewska-Piotrowska et al. 2009; Clift et al. 2010). Over the last decade, abiotic factors, including pH, ionic strength (IS), NOM

and ultraviolet (UV) light, have been shown to affect aggregation, bioavailability and toxicity of NMs in many studies (Schultz et al. 2014; Wormington et al. 2016; Ma et al. 2012; Zhu et al. 2014; Baalousha et al. 2008). The purpose of this chapter is to summarize some intrinsic properties affecting the environmental toxicity of NMs and highlight some of the results from recent research on the effects of environmental factors on the toxicity of nanomaterials.

Characteristics of NMs Modulating their Toxicity

The characterization of NMs themselves in both pristine and more importantly, environmentally relevant conditions is crucial in determining their potential for harm (Jiang et al. 2009). Since the early 2000s, nanotoxicologists have worked to identify some of the more important intrinsic properties including size, surface charge, shape and crystal structure, which play a critical role in determining the toxicity of NMs while more recently, recognition of environmental factors as modulators of toxicity (extrinsic properties), interacting with the intrinsic properties have become more studied (Jiang et al. 2009; Ren et al. 2016; Misra et al. 2014; Braydich-Stolle et al. 2009). An understanding and integration of all these factors are necessary when assigning hazard and risk to a particular material.

Size

The size of an NM modulates its interaction with living organisms, including an NM's internalization, mechanism and toxicity (Jiang et al. 2009; Shang et al. 2014). Endocytosis is an energy-dependent process used by cells to internalize molecules, and communicate with the biological environment and other cells (Shang et al. 2014; Oh and Park 2014; Felix et al. 2017a). Endocytosis is divided into four pathways including phagocytosis, macropinocytosis, clathrin-mediated endocytosis and caveolae-mediated endocytosis (Oh and Park 2014). Effects of primary size of NMs have been extensively studied in mammalian cell lines and while caution should be exercised when cross-reading to environmental receptors, we can use these findings as surrogates to inform our understanding of the effects of nanomaterials on non-mammalian biota. In a recent review of mammalian cell line nanoparticle uptake, results suggest that each NM has its optimal size for cellular uptake (Shang et al. 2014). For example, the 100 nm polystyrene nanoparticles have higher cellular uptake efficiency than 50, 200, 500 and 1000 nm while 50 nm has the lowest uptake rate of all the sizes tested (Oh and Park 2014). However, only a few studies have examined the effects of particle size on cellular uptake in fish cells (Felix et al. 2017a). A recent study demonstrated that smaller polyvinylpyrrolidone (PVP) coated silver (Ag) NPs had lower cellular accumulation in rainbow trout (*Oncorhynchus mykiss*) gill epithelial cell line (RTgill-W1), but a higher rate of

being transported through the multilayers of cells when compared to larger citrate coated Ag NPs (Farkas et al. 2011). Another paper from our laboratory revealed that clathrin-mediated endocytosis (CME), caveolae-mediated endocytosis (CavME) and macropinocytosis (MP) were all involved in the uptake of nominally 10 nm Nile-red-loaded NPs (<100 nm hydrodynamic diameter in L-15 medium) into RTgill cells, with CME being the dominant pathway (Felix et al. 2017a). This finding is consistent with the results from mammalian cell line studies which suggest that CME is the major uptake pathway for smaller NPs and cellular uptake shifted towards CavME and MP as NMs' size increases, and the uptake efficiency decreases when size is above a certain threshold (Shang et al. 2014; Rejman et al. 2004). However, given that only one size of NM was tested in RTgill cells, the independent effects of primary size on the pathway for uptake of NMs into fish cell lines has not been investigated.

With regard to testing of particles size in *in vivo* environmental models, there are only a few studies specifically and accurately address the issue of particle size. One study showed that *Daphnia magna* exposed to TiO_2 NPs with the primary size of 25 nm had the highest rate of immobilization when compared to animals exposed to smaller (10 nm) and larger (220 nm) sized particles. In that study, the varying toxicity was attributed to higher hydroxyl radicals generation in the intermediately sized materials (Wyrwoll et al. 2016). Another study designed to examine the effect of the size of Ag NPs demonstrated a size-dependent distribution and toxicity in rainbow trout (Scown et al. 2010). Ag NPs with a primary size of 10 nm had greater accumulation on the gills when compared to 35 and 600 nm Ag NPs. Furthermore, 10 nm Ag NPs significantly increased the gene expression of *cyp1a2* in the gills which may indicate up-regulation of oxidative metabolism due to external or internal oxidative stress (Scown et al. 2010). Similar trends were observed in adult zebrafish (*Danio rerio*) where citrate-coated Ag NPs with a primary size of 20 nm had significantly higher uptake through the gill and intestine, was associated with higher damage on the gills including fusion of secondary lamellae, hyperplasia and inflammation, and reduced Na^+/K^+ ATPase activity by 57% in the gill, significantly greater compared to the 21% reduction found in fish exposed to citrate-coated Ag NPs with a primary size of 100 nm (Osborne et al. 2015).

Surface Charge

Cell membranes on water contact surfaces such as gills or skin are negatively charged due to the presence of sialic acid-based glycosylation of membrane proteins (Ganguly et al. 2018; Varki and Schauer 2009). As a result, positively charged NMs have a much higher affinity to the outer epithelial membrane and can be taken up more efficiently than negatively charged and neutral NMs due to the electrostatic interaction between cells and these positively charged NMs (Ganguly et al. 2018; Kou et al. 2013; Iversen et al. 2011). For example, a study by Ganguly and colleagues suggested that the cellular uptake of positively charged Au NPs is

approximately five times higher than the uptake rate of negatively charge Au NPs (Ganguly et al. 2018). Similarly, in an *in vivo* study in *D. magna*, researchers demonstrated that enterocytes of the gut had significantly higher reactive oxygen species (ROS) production when exposed to positively charged Au NPs than negatively charged NPs at all three concentrations (1, 10 and 50 μg L^{-1}) tested (Dominguez et al. 2015). Their results also suggested that the levels of *glutathione S-transferase* (*gst*) and *heat shock protein 70* (*hsp70*) expression were significantly upregulated by positively charged particles at a concentration of 50 μg L^{-1} while negatively charged NPs did not cause any significant change in gene expression (Dominguez et al. 2015). Silva and colleagues reported that Ag NPs with negatively charged coating had lower median lethal concentration (LC_{50}) values on *D. magna* than positively charged and neutral Ag NPs (Silva et al. 2014). Altogether, this demonstrates that surface charge is a significant mediator of exposure and dose for aquatic organisms.

Shape

The shape of the NMs can affect zeta-potential, SSA and stability of given NMs, each of which has been demonstrated to alter NM toxicity (Jiang et al. 2009; Misra et al. 2014; Fu et al. 2014; Fabrega et al. 2011). Studies suggested that the shape of the NM can alter SSA which may bring out significant changes in SSA and dissolution behavior (Misra et al. 2014; Misra et al. 2012). For example, Misra et al. characterized CuO NPs in two different shapes including spheres (7 nm) and rods (7 nm × 40 nm) with the same length at one dimension. The point of zero charge (PZC, see section "Ionic Strength and pH" for details) of the sphere was higher than the rod, and this may be due to the higher SSA of the sphere. These authors also found that the dissolution of spherical CuO NPs (2.5%) was higher than the rod-shaped NPs (0.8%) over 7 days, which also can be explained by higher SSA of the sphere (Misra et al. 2014). The shape-dependent characteristic of NMs has been shown to alter their toxicity directly. In *in vivo* study in adult zebrafish, the LC_{50} value of spherical nickel (Ni) NPs with 60 nm diameter was 361 mg L^{-1}, three times higher than the LC_{50} value of dendritic Ni NPs with a similar 60 nm aggregated size. However, spherical Ni NPs had a much higher rate of uptake (~3 times greater) than dendritic NPs (Ispas et al. 2009). Similar studies on *D. magna* have also supported that differences in toxicity of NMs result from different shapes (Bacchetta et al. 2018; Nasser et al. 2016). In one study, long rod gold (Au) NPs (25 nm × 146 nm) caused a significantly higher mortality rate in *D. magna* neonates than both short rod Au NPs (25 nm × 60 nm) and spherical Au NPs (25 nm). The results also showed that both positively charged rods and spheres induced significant ROS generation in *D. magna*, but the ROS level returned to normal level 24 hours after being exposed to spherical NPs while ROS level remained high in *D. magna* exposed to short rod NPs after 24 hours (Nasser et al. 2016). Therefore, it is worth to consider and report the shape of NMs when investigating their toxicity.

Crystal Structure

Crystal structure has been reported to have an impact on both solubility/dissolution of NM and interaction of NMs with UV light, each of which has the potential to alter the toxicity of given NMs (Clement et al. 2013; Avramescu et al. 2017). The solubility and dissolution behavior of NMs play an essential role in the bioavailability, rate of uptake and toxicity of each type of NM (Schultz et al. 2014; Ren et al. 2016; Avramescu et al. 2017). With regard to crystallinity, TiO_2 NPs have three described crystal structure forms (anatase, rutile and brookite) but only anatase and rutile are natural forms, and each is widely used in various applications (Clement et al. 2013; Oi et al. 2016). Anatase TiO_2 NPs are more toxic to a variety of organisms, including mammalian cell lines, green algae and *D magna (*Braydich-Stolle et al. 2009*;* Clement et al. 2013*;* Menard et al. 2011*)*. A study on green algae *Chlorella* sp. showed that anatase TiO_2 NPs reduced the growth of algae by 75% at an arguably non-ecologically relevant nominal concentration of 1000 mg L^{-1} at pH 6.5 while rutile TiO_2 NPs did not have any significant impact on the growth at the same concentration (Ji et al. 2011). The median effective concentration (EC_{50}) value of immobility of *D. magna* exposed to 99.5% anatase TiO_2 with a primary size of 20 nm was 35.3 mg L^{-1}, and the EC_{50} value increased to over 100 mg L^{-1} when rutile TiO_2 NPs were added and the purity of anatase reduced to 70% (Menard et al. 2011). While these studies suggested that the higher toxicity of anatase TiO_2 NPs was due to its higher solubility (Clement et al. 2013; Avramescu et al. 2017), we suggest that the differences in toxicity may be more attributable to differences in crystallinity. Both anatase and rutile TiO_2 NPs are practically insoluble (less than 0.000066% dissolution rate) at or close to neutral pH (7) where these tests were conducted. While the study did show that anatase TiO_2 NPs had a higher dissolution (0.022% over 2 hours) than that rutile form (0.00016% over 2 hours), this was conducted in a solution with a very low pH (1.5) which is not ecologically or biologically relevant (Avramescu et al. 2017). We believe that a better explanation is that anatase form produces more ROS than rutile form, even under non-UV conditions, and that uptake of particles likely induced ROS mediated toxicity (Clement et al. 2013; Ji et al. 2011). Other studies have confirmed a role for crystallinity in superoxide radical ($O_2^{\bullet -}$) formation under both laboratory (non-UV) and UV conditions. In each case, rutile TiO_2 NPs (30 nm × 70 nm) have been shown to demonstrate lower photoactivity when compared to the crystalline anatase form (30–50 nm), resulting in substantially higher $O_2^{\bullet -}$ generation by the anatase form under UV light conditions (Buchalska et al. 2015). Therefore, it is essential to report crystal structure when studying the toxicity of NMs with more than one crystal structure.

Dissolution

Many studies have demonstrated that dissolution of metallic NMs and release of free metal ions either acted as the primary mechanism of toxicity to aquatic organisms or contributed to part of the toxicity, especially in an aqueous medium (Schultz et al. 2014; Ren et al. 2016). There is a substantial amount of literature on the mechanisms and mediation of various metals toxicity in aquatic animals (Paquin et al. 2000; Di Toro et al. 2001; Blewett and Leonard 2017). This chapter will not cover the specific aspects of metal toxicity per se but instead focus on the environmental properties affecting dissolution and subsequent toxicity. Dissolution depends on the intrinsic characteristics of the NMs including particle size, particle type, concentration and also specific environmental factors including pH, temperature and natural organic matter (NOM) (Schultz et al. 2014; Ma et al. 2013). Dissolution of many metallic NPs has been shown to increase as concentration decreases due to lower aggregation/increased dispersion at lower concentrations (Baalousha et al. 2016; Sikder et al. 2018). As particle size decreases, the SSA increases exponentially, which results in higher percentage of atoms exposed to the environment and therefore a higher dissolution rate (Schultz et al. 2014; Ma et al. 2013). In a study on the size-dependent dissolution of zinc oxide (ZnO) NPs, a particle with the smallest primary size (4 nm) had the highest dissolution rate (5.7%) comparing to 15 nm (2.2%) and 241 nm (1.0%) at pH 7.5 (Bian et al. 2011). Environmental pH has almost no impact on the dissolution of TiO_2 NPs while that of ZnO NPs is highly dependent on the environmental pH (Wormington et al. 2016; Bian et al. 2011; Omar et al. 2014). CeO_2 NPs are relatively insoluble in the water at pH > 5, but their dissolution becomes pH-dependent when environmental pH is lower than 4.5 (Dahle et al. 2015). Studies have shown that increasing temperature and low pH tend to enhance the dissolution rate of metallic NMs (Schultz et al. 2014; Ren et al. 2016) while NOM can increase dissolution by donating chelating agents for metal ions or reduce dissolution by preventing interaction between particles and water molecules when adsorption onto the surface of the particles (He et al. 2018; Ma et al. 2013). Two studies on ZnO NPs dissolution and toxicity demonstrated that iron (Fe) doped ZnO NPs had a significantly lower dissolution rate as Fe percentage increasing. They also concluded that ZnO NPs reduced the hatching success of zebrafish embryos to approximately 40% while 1%, 4% and 10% Fe-doped ZnO NPs with a similar particle size as ZnO NPs increased hatching success to over 65% (Xia et al. 2011; George et al. 2009). Therefore, it is necessary to definitively address in each study to potential confounding issues of the presence of separate toxicity of dissolved ions compared with the toxicity of NP in the presence of dissolved ions and by subtraction, address the toxicity of the NP itself. Authors of papers are highly encouraged to report dissolution rates when describing the exposure toxicity of any given NMs.

Environmental Factors Affecting the Toxicity of Nanomaterials

Once released into the environment, NMs and their subsequent biological effects are known to be modulated by various environmental factors including ionic strength (IS), pH, natural organic matter (NOM) and ultraviolet (UV) light, each of which can cause distinct physicochemical changes (e.g., agglomeration/aggregation, change in surface charge and electronic excitation) that have the potential to alter bioavailability and the toxicity of NMs (Schultz et al. 2014; Jiang et al. 2009; Badawy et al. 2010).

Ionic Strength and pH

While the pristine sizes of NMs (as discussed above) play distinct roles in diffusivity and transport, the actual size as demonstrated in the environment during the exposure period also plays an integral role in determining their distribution and bioavailability to aquatic organisms (Schultz et al. 2014). The stability of NMs can be achieved mainly be two mechanisms, electrostatic stabilization and steric stabilization (Fig. 1) (Schultz et al. 2014; Jiang et al. 2009; Badawy et al. 2010; Sperling and Parak 2010). However, sterically stabilized uncharged polymer-coated NMs are relatively insensitive to ionic strength and pH (Badawy et al. 2010; Sperling and Parak 2010). A study published in 2010 demonstrated that the zeta- potential and hydrodynamic diameter (HDD) of PVP coated Ag NPs did not significantly change over the pH range from 3 to 9 and ionic strength of 10 and 100 mM (Badawy et al. 2010). Therefore, electrostatically stabilized NMs are the focus of this section. Derjaguin Landau Verwey Overbeek (DLVO) theory states that the size and stability of NMs in suspension are affected by the sum of van der Waals forces (attractive forces) and electrostatic forces (repulsive forces) (Schultz et al. 2014; Zhang et al. 2009; Jiang et al. 2009; Yin et al. 2014). Aggregation occurs when the attractive forces dominate repulsive forces and the opposite is true for disaggregation (Mitrano et al. 2015; Zhu et al. 2014; Baalousha et al. 2008). Electrostatic repulsive forces are generated by the electrical double layers (Fig. 1) of particles interacting with each

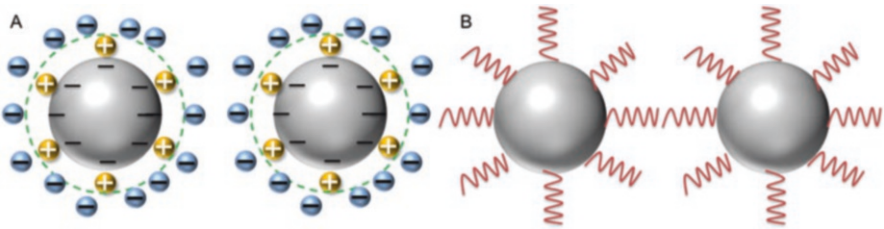


Fig. 1 Electrostatic stabilization (**a**) and steric stabilization (**b**) of NMs

other (Jiang et al. 2009). IS and pH are two important abiotic factors that have effects on NM agglomeration by affecting their electrical double layer (EDL) (Zhu et al. 2014; Jiang et al. 2009; Omar et al. 2014). The repulsive energy generated by the EDL is a function of surface charge (zeta potential) and the thickness of EDL (Jiang et al. 2009). Studies have shown that high IS in solution results in a reduction of the thickness of EDL and subsequent greater interaction between particles (Schultz et al. 2014; Jiang et al. 2009; Badawy et al. 2010; Yin et al. 2014). The higher IS reduces the electrostatic repulsive forces by shielding or neutralization which will increase agglomeration (Schultz et al. 2014; Jiang et al. 2009; Badawy et al. 2010; Yin et al. 2014). In general, the toxicity of an NM is usually reduced when there is a significant aggregation of the NM into larger agglomerates (Wyrwoll et al. 2016; Kim et al. 2013; Römer et al. 2011; Römer et al. 2013). A few papers have linked increasing ionic strengths to both increase agglomeration with direct resulting lower relative toxicity (Wyrwoll et al. 2016; Kim et al. 2013). For example, EC_{50} value of TiO_2 NPs on the immobility of *D. magna* was increased from 1.28 mg L^{-1} (10 nm) and 0.53 mg L^{-1} (25 nm) in a medium with IS of 865 μM to 2.9 mg L^{-1} and 1.1 mg L^{-1} in a medium with IS of 8653 μM respectively (Wyrwoll et al. 2016). Similar trends were also found in Ag NPs. The lowest observed adverse effect concentration (LOAEC) of immobility was estimated to be 11.25 μg L^{-1} when *D. magna* were exposed to Ag NPs (7 nm) while the LOAEC decreased to 2.5 μg L^{-1} when *D. magna* were exposed to the same NPs at the same concentration but in the tenfold diluted media (Römer et al. 2013). A study on the effects of Ag NPs on zebrafish embryos demonstrated that Ag NPs suspension with both primary sizes of 20 and 100 nm had significantly lower LC_{50} values in ultrapure water and the media with IS of approximately 187.5 μM than the media with IS of about 22.9 mM. The EC_{50} of mortality and malformation for citrate-stabilized Ag NPs suspension was also significantly lower in the media with IS of 22.9 mM (Kim et al. 2013).

Environmental pH also plays an important role in controlling the zeta potential, resulting in aggregation/agglomeration, and therefore toxicity of given NMs (Jiang et al. 2009; Badawy et al. 2010). Zeta potential measures the electric potential at a certain distance from the plane of shear (Badawy et al. 2010) and it is not the same as surface charge (Fig. 2) (Jiang et al. 2009). For a constant medium ionic strength, the zeta potential, surface charge and HDD of a given NM are significantly affected by the pH of the aqueous medium (Baalousha et al. 2008; Jiang et al. 2009; Badawy et al. 2010). The PZC is the pH of a given medium when NM has zero net surface charge. Therefore, the absence of electrostatic repulsive force prevents disagglomeration and NMs will settle out of suspension eventually (Jiang et al. 2009; Omar et al. 2014; Badawy et al. 2010). As pH becomes progressively lower than PZC, NMs have a more positively charged surface. On the other hand, NMs are more negatively charged as pH is progressively higher than the specific PZC (Zhu et al. 2014; Jiang et al. 2009; Omar et al. 2014). In general, an NM with the zeta potential higher than +20 mV or lower than −20 mV is considered stable and its agglomeration rate is low or even close to zero (Zhu et al. 2014; Badawy et al. 2010). In an aquatic environment where ionic strength is low and pH is far away from the NM's

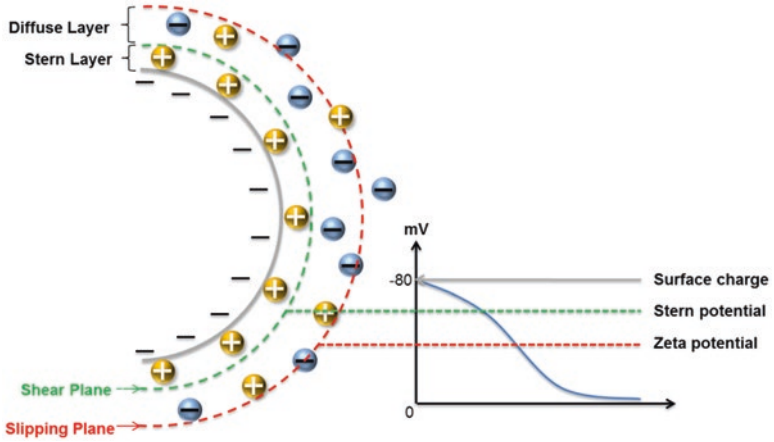


Fig. 2 The simplified model of electrical double layer at a negatively charged NM's surface in an aqueous medium

PZC, NMs will have generally low agglomeration rate and are more likely to remain suspended as individual particles or small agglomerates in the medium (Zhu et al. 2014; Jiang et al. 2009). As discussed above (see section "Surface Charge"), cell membranes are negatively charged (Ganguly et al. 2018; Varki and Schauer 2009). The alterations of environmental pH below PZC will result in positively charged NMs which leads to a higher binding potential to respiratory surfaces including gills and skin. Accumulation of NMs on the surface of gills can result in simply irritation and mucus hypersecretion which have a negative impact on gas transport, osmoregulation and alter the uptake of molecules across the biological membrane through normal endocytotic processes (Garcia-Reyero et al. 2015). Therefore, pH affects the toxicity of NMs through both altering their aggregation/agglomeration and altering the interaction of the individual and agglomerated materials with respiratory surfaces. For example, an *in vitro* study reported that 24-hour aged ZnO NPs with lower aggregation size had a significantly higher negative impact on the mitochondrial activity of RAW 264.7 cell line at concentrations of 10, 15 and 20 μg mL^{-1}, and induced higher generation of ROS at a concentration of 10 μg mL^{-1} than ZnO NPs with higher aggregation size (Tripathy et al. 2014). An in vivo study reported that 48 hours- EC_{50} value of immobility of *D. magna* exposed to citrate-coated Ag NPs was significantly higher (1.5 times) in the environment with pH of 8 than that in pH of 6.5 when there was no significant difference in the Ag^{+} concentration. It also demonstrated that lower pH significantly reduced the number of offspring produced during the exposure, and increased mortality from 30% at pH of 8 to 90% without the presence of NOM and from 20% at pH of 8 to 40% with the presence of NOM after exposed to 78 μg L^{-1} NPs for 21 days (Seitz et al. 2015). In whole animal studies examining the effects of IS and pH on aggregation and the toxicity of NMs, researchers have often used NMs with different concentrations and/or they compared NMs to their bulk form counterparts to achieve different aggregation size/

rate (Sharma 2009; Ates et al. 2013; Wong et al. 2010). However, in many studies, researchers have failed to characterize NMs in the environmental test conditions, using the characterization of their bulk/pristine forms as a guide. This has perhaps led to simplistic interpretations of the principle toxicological drivers. In media with biological organisms, NMs can assume different/new physiochemical properties which should be considered to properly interpret these ecotoxicity studies. In many cases, the aggregation/agglomerations and resulting changes in concentration, and/or significant changes in ion concentration were not accounted for, inevitably acting as confounding factors (Schultz et al. 2014; Sharma 2009; Wong et al. 2010). In general, NMs with lower aggregation size/rate are considered to have more toxic potential largely due to the greater surface area available for biological interactions and increased potential for transport into the animal (Schultz et al. 2014; Bystrzejewska-Piotrowska et al. 2009; Sharma 2009; Wong et al. 2010). The combination of low agglomeration rate, small size and high SSA will increase the bioavailability of the NMs to interact with the living organism in the water column (Schultz et al. 2014). An aquatic environment with higher ionic strength and/or pHs close to PZC will enhance the agglomeration of NMs resulting NMs settling out and concentrating at the bottom of the test vessel or in the sediment. In this case, risk is transferred from pelagic to benthic organisms and negatively buoyant embryos (Schultz et al. 2014; Keller et al. 2010; Baalousha et al. 2008). As mentioned in section "Shape", pH can also have significant effects on the dissolution of specific metallic NMs resulting in changes in dissolved ion concentration which potentially can mediate some of the toxicity of metallic NMs (e.g. ZnO NPs and Ag NPs) (Scown et al. 2010; Ma et al. 2013). In summary, environmental ionic strength and environmental pH can have a significant impact on the aggregation, dissolution, distribution, bioavailability and the potential target organisms of NMs.

Ultraviolet Light

Solar radiation has important effects on every life on Earth (Williamson et al. 2014). Its UV component is divided into three groups based on wavelength, including UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm) (Williamson et al. 2014; Safari et al. 2015). The shorter wavelength UVC and UVB are the most damaging, but all the UVC is blocked and the majority of UVB is absorbed by the stratospheric ozone layer. Large amounts of UVA can pass through the stratospheric ozone layer and reach the surface and therefore this type of UV light has more environmental relevance (Williamson et al. 2014). The majority of nanotoxicity studies are performed in laboratory conditions (Schultz et al. 2014; Mitrano et al. 2015), where fluorescent lamps used emit negligible amounts of UVA and UVB radiation (Safari et al. 2015). However, many NMs released into the environment either are wide band-gap semiconductors or have semiconductor properties (Fu et al. 2014). Studies have shown that these NMs can absorb energy including photon energy (light) and phonon energy (heat). When the energy input is at or above the band gap

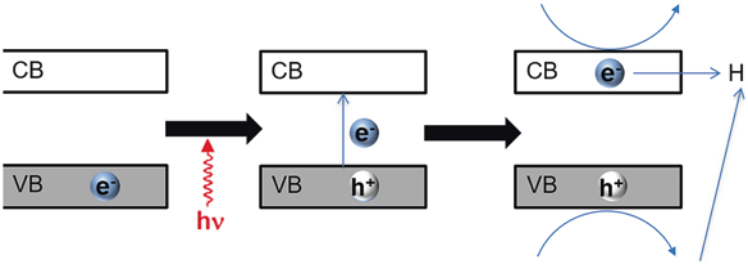


Fig. 3 The generation of reactive oxygen species (ROS) on the surface of semiconductor NMs under UV radiation exposure and the scavenging activity of humic acid (HA). Valence band (VB), conduction band (CB)

between the valence band and the conduction band, electron on valence electrons will absorb enough energy to jump to the conduction band becoming a free electron and leave a positively charged hole (Fig. 3) (Ma et al. 2012; Fu et al. 2014). This results in an electron-hole pair, which is able to react with oxygen and water molecules in the environment to produce reactive oxygen species (ROS) including hydroxyl radicals (•OH), superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) (Ma et al. 2012; Fu et al. 2014). ROS are highly reactive due to their unpaired electron and are able to cause cellular damage when overwhelming a cell/organism's antioxidant defense system (Sharma 2009; Dahle and Arai 2015; Apel and Hirt 2004; Riley 1994). ROS and RNS are naturally derived substances in normal metabolism. For example, NO• and H_2O_2 play an important role in cellular signaling and regulating apoptosis (Ježek and Hlavatá 2005). They are also involved in the recognition process by macrophages and neutrophils in the innate immune system and combating bacterial infections by immune cells (Riley 1994; Di Meo et al. 2016; Kohchi et al. 2009). Similarly, superoxide radical ($O_2^{\bullet -}$) is generated due to incomplete reduction in the mitochondria during oxidative phosphorylation (Fu et al. 2014; Ježek and Hlavatá 2005). Therefore, an antioxidant defense system has evolved to both regulate endogenous ROS/RNS production and also mitigate ROS/RNS caused by external stress (Riley 1994). Antioxidant enzymes such as catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD), and antioxidants like glutathione are all part of this process (Riley 1994; Ježek and Hlavatá 2005; Di Meo et al. 2016). Nevertheless, when band gap semiconductor NMs are exposed to UV light, many studies have shown they generate ROS causing excess oxidative stress. When the systemic manifestation of ROS overwhelms an organisms' antioxidant defense capacity, the negative effects of ROS and NOS include excess lipid peroxidation and protein carbonylation which can be used a marker of excess ROS/NOS exposure (Ren et al. 2016; Wyrwoll et al. 2016; Fu et al. 2014).

Most studies on phototoxicity of semiconductor NMs have focused on metal oxide nanoparticles, especially titanium dioxide nanoparticles (TiO_2 NPs), in a variety of species (Ma et al. 2012; Wyrwoll et al. 2016; Fu et al. 2014; Bar-Ilan et al. 2012). Phytoplankton are the foundation of the food web in the aquatic environment where they contribute to over 100 million tons of carbon cycle every day as the

dominant primary producers (Miller et al. 2012; Behrenfeld et al. 2006). Phytoplankton rely on solar energy for photosynthesis so they may be vulnerable to photo-inducible NMs (Wyrwoll et al. 2016; Miller et al. 2012; Behrenfeld et al. 2006). A study reported that TiO_2 NPs at concentrations of 1–3 mg L^{-1} significantly suppressed the population growth rate from 50% to almost 100% in three marine phytoplankton species under UV (Miller et al. 2012). Locomotion is important to the aqueous organism for feeding and avoiding predation so the decreased mobility will reduce their ability to survive in nature. Wyrwoll et al. reported that TiO_2 NPs with 25 nm primary size had higher phototoxicity on *D. magna* under UV exposure than laboratory light. The EC_{50} of immobility was 0.53 mg L^{-1} (nominal concentration) under UV exposure while *D. magna* did not show any immobility at the concentration of 100 mg L^{-1} under laboratory light at 48-hour exposure (Wyrwoll et al. 2016). This studies also suggested a significant increase in the production of •OH and $O_2^{\bullet-}$ by UV light may be the cause of the phototoxicity of TiO_2 NPs (Wyrwoll et al. 2016). Studies also showed UV light increased the mortality of *D. magna* during 48-hour exposure ($LC_{50} = 29.8$ µg L^{-1} under UV stimulation vs. $LC_{50} > 500$ mg L^{-1} under laboratory light) and reduced growth rate in other marine phytoplankton species exposed to TiO_2 NPs at a concentration of 1 mg L^{-1} and above under UV exposure (Ma et al. 2012; Miller et al. 2012). Therefore, any change in abundance and biomass of phytoplankton and primary consumer in food chain caused by co-exposure of semiconductor NMs and UV light may have a significant impact on the stability of the ecosystem.

The early development stage of organisms is a complex process and very vulnerable to chemical disturbance (Fig. 4) (Bar-Ilan et al. 2012). A study done by Bar-Ilan et al. demonstrated that UV illumination significantly reduced survival rate and increased the incidence of malformation, including yolk sac edema, pericardial edema, spine stunting and tail abnormality, of zebrafish embryo exposed to TiO_2 NPs for 92 hours starting from 4 hours post-fertilization (hpf). This may be explained by the dramatic increase in •OH production in the water by TiO_2 NPs under UV illumination (Bar-Ilan et al. 2012). Phototoxicity of NMs has been reported in other species at different life stages. The LC_{50} of juvenile Japanese Medaka exposed to TiO_2 NPs for 96 hours was significantly decreased from the nominal concentration of 155 mg L^{-1} under laboratory light to 2.19 mg L^{-1} under UV light (Ma et al. 2012). Similar results were reported by Ma et al. in *D. magna*. The 48-hour LC_{50} value decreased from over 500 µg L^{-1} under laboratory light to 29.8 µg L^{-1} under UV light (Ma et al. 2012).

For less well-studied semiconductor NMs, Zhang et al. examined the effects of CeO_2 NPs on the lipid peroxidation and morphological alternations in gills of cardinal tetra. CeO_2 NPs under the stimulation of UV light significantly induced lipid peroxidation in gills at medium (2 mg L^{-1}) and high (5 mg L^{-1}) nominal concentrations (Zhang et al. 2018). Moreover, co-exposure of CeO_2 NPs and UV light caused the medium to serve damage on gills including an increase in interlamellar cell mass, fusion of lamellae, hyperplasia of primary lamellae and lifting of respiratory epithelium (Fig. 6) (Zhang et al. 2018).Authors concluded that observed photo-toxicity of CeO_2 can be explained by the increase in the

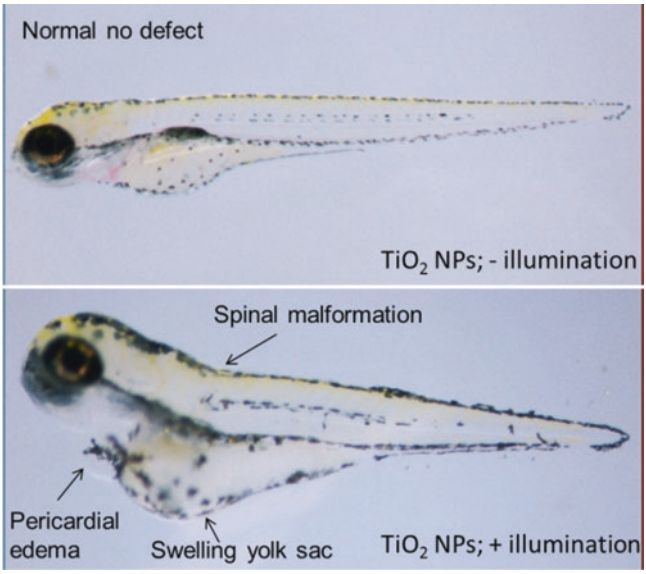


Fig. 4 Morphology of zebrafish embryo at 120 hpf after exposure to 1 mg L^{-1} TiO_2 NPs (25 nm) under UV light (+ illumination) and laboratory light (− illumination)

generation of hydroxyl radical under UV light (Zhang et al. 2018). Therefore, toxicity studies on NMs, especially band gap NMs, should include UV stimulation to enhance the understanding of the potential phototoxic effects in an environmental realistic condition.

Natural Organic Matter

Natural organic matter (NOM) is an anionic heterogeneous matrix of carbon-based compounds formed from the microbial decomposition of plants, algae and animals, and their waste products (Grillo et al. 2015). The composition of degraded products varies between different origins and shows temporal changes (Grillo et al. 2015; Dahle and Arai 2015) and therefore, in this review, NOM is referred to as a bulk constituent primarily dominated by humic and fluvic acids. For a more complete understanding of the role(s) or different constituents of NOM on the interaction with NMs, the reader is referred to some excellent papers by Nason et al. (Nason et al. 2012) and Gallego-Urrea et al. (Gallego-Urrea et al. 2014) NOM is present in both terrestrial and aquatic environments, and humic acid (HA) and fulvic acid (FA) are two of the most abundant compositions in many origins of NOM (Grillo et al. 2015). NOM is divided into a hydrophobic portion which contains aliphatic carbon and a hydrophilic portion which

is composed of humic-like substances (Grillo et al. 2015; Levchuk et al. 2018). The carboxyl group (-COOH) in the hydrophobic portion and phenolic group in the hydrophilic portion make NOM negatively charged at the pH value of natural water (Grillo et al. 2015; Levchuk et al. 2018). Aggregation/disaggregation mainly depends on the environmental pH, environmental divalent ion concentration, concentration ratio of NOM to NMs and PZC of NMs (Omar et al. 2014). The adsorption of low molecular mass NOM onto NMs' surface forms an ecocorona, which will increase the electrostatic repulsion between NMs due to more negative zeta potential or increase steric repulsion caused by adsorption of NOM on the surface (Jiang et al. 2009; Sharma 2009) to reduce the aggregation and increase the stability of NMs in the water column (Zhang et al. 2009; Quik et al. 2010; Van Hoecke et al. 2011) when the concentration of divalent ions (i.e. Ca^{2+} and Mg^{2+}) is lower than the critical coagulation concentration (Fig. 5) (Jiang et al. 2009; Omar et al. 2014). However, NOM can also cause bridging effects to enhance the aggregation in the environment with high ionic strength and the presence of divalent ions (Fig. 5) (Zhu et al. 2014; Baalousha et al. 2008). Studies have shown that the formation of an ecocorona can modulate the toxicity of NMs in a variety of aquatic species (Grillo et al. 2015; Schultz et al. 2014; Ma et al. 2013; Gao et al. 2009). The EC_{50} of the growth rate of unicellular green algae *P. subcapitata* was determined to be 1.24 mg L^{-1} in CeO_2 NPs and 0.27 mg L^{-1} in TiO_2 NPs. The presence of Suwannee River NOM at a concentration of 8 mg L^{-1} increased EC_{50} values (> 30 mg L^{-1}) of both NPs way above ecologically relevant concentration (Cerrillo et al. 2016). The presence of NOM from three different origins has been reported to significantly increase LC_{50} values of Ag NPs, especially copper nanoparticles (Cu NPs), on *Ceriodaphnia dubia (*Gao et al. 2009*)*. This reduction in toxicity may be explained through three mechanisms (1) the adsorption of NOM onto NMs' surface can reduce the direct interaction between NMs' surface and organisms, decrease the cellular uptake of

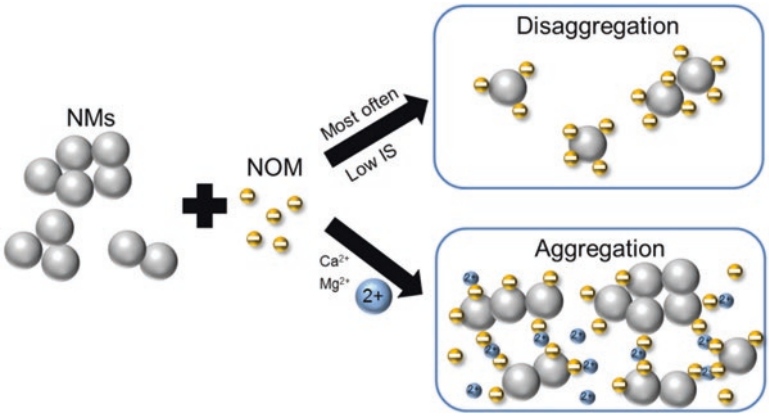


Fig. 5 Modified schematic particle stability diagram of nanomaterials interacting with natural organic matter and divalent ions. (Grillo et al. 2015; Zhu et al. 2014; Omar et al. 2014)

NMs, and reduce the bioavailability of NMs to lower the toxicity of NMs (Dahle and Arai 2015; Van Hoecke et al. 2011; Westmeier et al. 2016); (2) the adsorption of NOM can decrease the interaction between metallic NMs' surface and water molecules to reduce the dissolution which will decrease the toxicity caused by metallic ions (He et al. 2018; Ma et al. 2013; Quigg et al. 2013); (3) free ions released from NPs can be complexed by the presence of NOM and therefore the bioavailability of free ions is reduced (Ma et al. 2013). On the other hand, the adsorption of high molecular mass NOM and increased aggregation due to charge neutralization and bridging effects will favor the removal of NMs from the water column and increase the bioavailability to the organisms in the sediments (Baalousha et al. 2008; Omar et al. 2014; Quigg et al. 2013). Therefore, the presence of NOM not only affects the distribution and bioavailability of NMs in the environment but also plays an important role in determining the toxicity of NMs in organisms.

As discussed in section "Ultraviolet Light", UV light can induce the toxicity of NMs, especially semiconductor NPs. In recent years, NOM has been found to have protective properties against phototoxicity of NMs (Schultz et al. 2014; He et al. 2018; Li et al. 2016). NOM is able to attenuate UV light to reduce the amount and the intensity of UV light received by NMs (Wormington et al. 2016). Humic acid (HA) present in many NOM is considered to be an electron acceptor pool (Grillo et al. 2015; He et al. 2018; Heitmann et al. 2007; Scott et al. 1998). Studies have shown that NOM containing humic acid can accept electrons excited by UV light and quench the production of ROS which can reduce the photo-toxicity of NMs (Fig. 3) (Grillo et al. 2015; Schultz et al. 2014; He et al. 2018; Wormington et al. 2016). For example, Wormington et al. demonstrated that NOM at the concentration of 4 mg L^{-1} reduced the morality of *D. magna* from over 90% (0 mg L^{-1} NOM) to less than 10% when exposed to 1.5 mg L^{-1} TiO_2 NPs under UV light for 48 hours. Such a significant decrease in toxicity can be partially explained by the reduced production of H_2O_2 equivalents measured in the media at the presence of NOM due to its ROS quenching ability (Wormington et al. 2016). In addition, a study on UV-induced toxicity of CeO_2 NPs in cardinal tetras mentioned in section "Ultraviolet Light" also showed the protective property of NOM from Rio Negro Amazon water (Zhang et al. 2018). This study demonstrated that the presence of NOM significantly inhibits the production of hydroxyl radicals in the water under the co-exposure of CeO_2 NPs and UV light. This lead to the reduction of lipid peroxidation to control level in the gills and decreased (but not necessarily eliminated) the severity of gill damage caused by co-exposure of CeO_2 NPs and UV light (Zhang et al. 2018). These results showed the protective property of NOM against the photo-toxicity of NMs (Fig. 6).

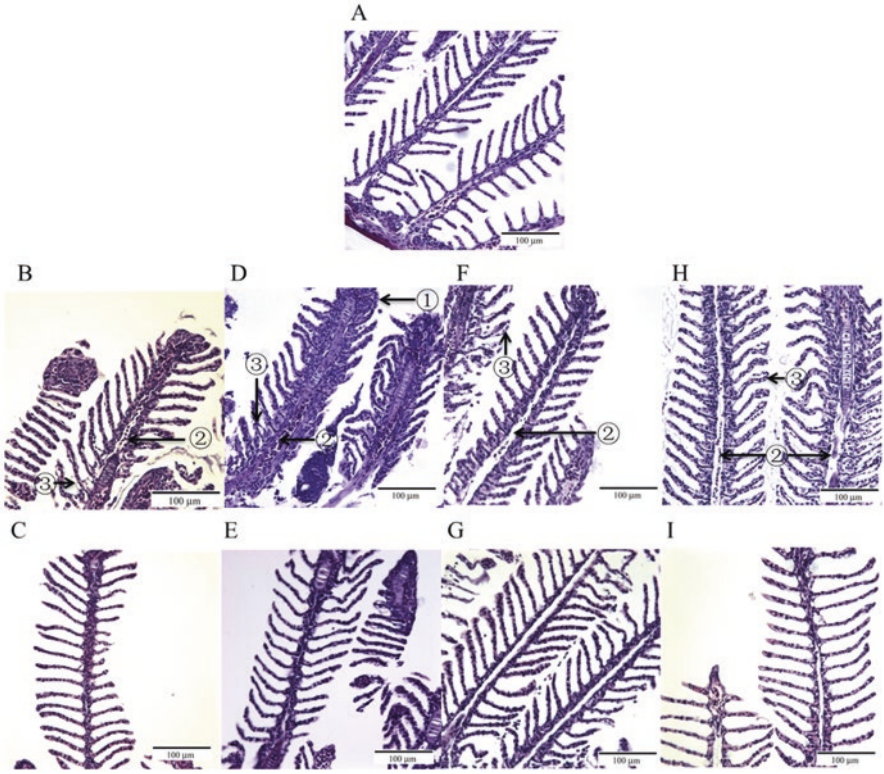


Fig. 6 Gill morphology in cardinal tetras after 48-hour co-exposure to CeO2 NPs and UV light (haematoxylin and eosin stain, ×400) at various nominal concentrations. (**a**) Control (0 mg L^{-1} NPs in Rio Negro water, no UV); (**b**) 0 mg L^{-1} NPs in ddH2O under UV light; (**c**) 0 mg L^{-1} in Rio Negro water under UV light; (**d**) 0.5 mg L^{-1} NPs in ddH2O under UV light; (**e**) 0.5 mg L^{-1} in Rio Negro water under UV light; (**f**) 2 mg L^{-1} NPs in ddH2O under UV light; (**g**) 2 mg L^{-1} in Rio Negro water under UV light; (**h**) 5 mg L^{-1} NPs in ddH2O under UV light; (**i**) 5 mg L^{-1} in Rio Negro water under UV light. Fusion of several lamellae (①). Hyperplasia of primary lamellae (②). Lifting of the respiratory epithelium and oedema (③). (Zhang et al. 2018)

Review of Recent Research on Ecotoxicity of Nanomaterials

The toxicity studies on nanomaterials, especially metal and metal oxide NMs, have been extensively researched for many years (Shaw and Handy 2011). In recent years, more and more *in vivo* studies have started to focus on conducting experiments in ecologically relevant conditions. Studies mentioned above and listed in Table 1 are a non-definitive summary of recent publications, showing that NMs have a wide range of impacts on a variety of organisms including phytoplankton, aquatic invertebrates, reptiles and fish. Semiconductor metal oxide NMs are the most extensively studied NMs. However, their phototoxicity and the effects of NOM on their phototoxicity have not attracted significant attention until the past few years.

Table 1 Selected toxicity studies of nanoparticles on a variety of organisms. The concentrations in the exposure conditions are nominal concentration useless told otherwise

NMs	Species	Exposure conditions	Effects	Characterization	Study
ZnO	*D.magna*	0.25–10 mg L^{-1} for 24 h and 48 h	48 h LC_{50} of ZnO NPs: 1.02 mg L^{-1} (30 nm), 1.10 (80-100 nm) and 0.89 (>200 nm) Dose dependent decrease in 24 h feeding rate in all three sizes with increasing concentration	Brunauer-Emmett-teller (BET) method (surface area) Transmission electron microscopy (TEM, primary size) Dynamic light scattering (DLS, HDD and zeta-potential) Dissolution	Lopes et al. (2014)
		0–0.75 mg L^{-1} for 21d	Dose and size-dependent effects on reproduction: (1) total number of neonates decreased as primary particle size increase; (2) particles with smaller primary size had a greater reduction in neonate production.		
	Danio rerio (AB adult)	0–50 mg L^{-1} for 24, 48, 72 h and 96 h	Time and dose-dependent effects on mortality. 96 h LC_{50} of ZnO NPs: 3.969 mg L^{-1}; bulk: 2.525 mg L^{-1} Dose-dependent increase in •OH production only in ZnO NPs under UV exposure with increasing concentration	Scanning electron microscopy (SEM) and SEM: 80 ± 49 nm (nano) and 454 ± 191 (bulk) DLS: aggregated size of 449–1381 nm (nano) and 811–742 (bulk)	Yu et al. (2011)
	Danio rerio (AB embryo-larval)	0–100 mg L^{-1}	Hatching rate decreased as the concentration of ZnO NPs increase (dose-dependent) Dose-dependent increase in SOD activity as the concentration of ZnO NPs increase Production of malondialdehyde (MDA) and generation of ROS was significantly induced at 20, 50 and 100 mg L^{-1}	SEM and TEM Inductively coupled plasma-mass spectrometer (ICP-MS, measure dissolved Zn^{2+} concentration) DLS (diameter distribution)	Zhao et al. (2013)

NMs	Species	Exposure conditions	Effects	Characterization	Study
TiO_2	*D.magna*	0, 0.01, and 1 mg L^{-1} for 48 h and 21d	Growth rate and reproduction ability were both significantly decreased at concentration of 0.01 and 0.1 mg L^{-1} after 21d Amylase and esterase activity were significantly reduced at concentrations of 0.01 and 0.1 mg L^{-1} after 48 h exposure while catalase activity was increased	X-ray fluorescence spectromicroscopy (m-XRF, detect and measure TiO_2 content)	Fouqueray et al. (2012)
	Danio rerio (AB embryo)	0, 1, 10 and 100 mg L^{-1} for 96 h	Hatching rate and the length of larvae was significantly reduced at concentrations of 10 and 100 mg L^{-1} under UV exposure GST activity was significantly decreased at 10 mg L^{-1} under UV light	DLS (HDD and zeta-potential)	Clemente et al. (2014)
	Danio rerio (AB embryo)	0, 0.1, 1 and 10 mg L^{-1}	Uncoated TiO_2 induced production of hydroxyl radicals in the water at the concentrations of 1 and 10 mg L^{-1}, increased lipid peroxidation and total glutathione level at all three treatment groups, and upregulated *glutathione peroxidase 1a* and *superoxide dismutase 2* gene expression under UV exposure	TEM DLS (HDD and zeta-potential)	Felix et al. (2017b)
	Oryzias latipes (embryo-larval)	0, 0.03, 0.07, 0.3, 0.7, 3, 7, and 14 mg L^{-1} for 17d	Premature hatching at 0.3, 0.7, 3, 7, and 14 mg L^{-1} (>55.3% comparing to 2.6% in control) Swimming activity and gill ventilation rate were significantly reduced in all TiO_2 NPs exposed groups.	DLS (HDD and zeta-potential)	Paterson et al. (2011)

(continued)

Table 1 (Continued)

NMs	Species	Exposure conditions	Effects	Characterization	Study
CeO_2	*Daphnia pulex & Daphnia similis*	0, 0.1, 1, 10, 50 and 100 mg L^{-1} for 96 h	48 h-EC_{50} of immobility for *D. pulex* was 91.79 mg L^{-1} while EC_{50} for *D. similis* was 0.26 mg L^{-1} 96 h-EC_{50} of immobility for *D. pulex* was 0.78 mg L^{-1} while EC_{50} for *D. simili* was lower than 0.1 mg L^{-1} Swimming velocity was significantly reduced in both species at 1, 10 and 100 mg L^{-1}. *D. simili* is more sensitive than *D. pulex* and dose-dependent	TEM Micro-X-ray fluorescence analytical microscope (measure Ce distribution in organisms)	Artells et al. (2013)
	Xenopus laevis	0–10 mg L^{-1} for 96 h	*Xenopus* had 35% mortality at 10 mg L^{-1} and growth was significantly reduced at 1 and 10 mg L^{-1}	DLS (HDD and zeta-potential) TEM	Bour et al. (2015)
	Danio rerio (AB larvae, 120hpf)	25 mg L^{-1} for 6 h with multiple impulse exposure	CeO_2 nanorods with an aspect ratio greater than 100 resulted in significant damage gastrointestinal tract including enterocytes desquamation, and had a negative impact on development such as reduced length and weight of larvae	TEM (measure primary length and diameter) DLS (HDD and zeta-potential) Inductively coupled plasma optical emission spectrometry (ICP-OES, measure Ce content)	Lin et al. (2014)

NMs	Species	Exposure conditions	Effects	Characterization	Study
Ag	*Danio rerio* (AB embryo and adult)	Ag NPs with PVP/PEI coating at 0, 0.01, 0.025, 0.05, 0.075 and 0.1 mg L^{-1} from 0 to 120 hpf	120 h- LC_{50} was 0.057 mg L^{-1} while (coating was 5.42 mg L^{-1}) Hatching success was significantly reduced at 0.05, 0.075 and 0.1 mg L^{-1} at 120 hpf Exposure to 0.05, 0.075 mg L^{-1} NPs significantly induced malformations including pericardial edema, yolk sac edema and spine curvature Exposure to NPs significantly decreased embryo production by at least 54.08% and increased the percentage of malformed embryos	DLS (HDD and zeta-potential) TEM Dissolution	Orbea et al. (2017)
	Oncorhynchus mykiss (juvenile)	40 μg L^{-1} for 96 h	Ag NPs significantly reduced immunoefficiency of leucocytes Ag NPs showed similar impact on cyclooxygenase activity as $AgNO_3$ and lower effects on metallothionein level and DNA damage in liver	TEM (gill image) Electron-dispersive X-ray analysis (EDS) DLS (HDD and zeta-potential) ICP-MS	Bruneau et al. (2016)

(continued)

Summary and Conclusions

In recent years, more and more new NMs have been created with the help of rapid development of nanotechnology, including NMs with magnetic properties for drug delivery and material retrieval, multifunctional nanohybrids by conjugating two or more NMs and pesticides with nano-based formulations to reduce the application rate and minimize their environmental impacts (Schultz et al. 2014; Bystrzejewska-Piotrowska et al. 2009; Rodrigues et al. 2013; Aich et al. 2014). The development of NMs certainly provides benefits in many areas but also brings new threats to the environment and challenges to toxicologists. The increased production of NMs will inevitably lead to more NMs released into the environment by a variety of routes (Mitrano et al. 2015; Saleh et al. 2015). The beneficial properties of NMs in the products may cause certain risks to non-target organisms under various environmental conditions. Based on current toxicological results, environmental factors have a significant impact on the distribution, bioavailability and toxicity of NMs (Schultz et al. 2014; Wormington et al. 2016; Ma et al. 2012; Zhu et al. 2014; Baalousha et al. 2008). However, many toxicity studies on NMs, especially semiconductor NMs, have been done in a laboratory condition without UV exposure and NOM, which research has demonstrated this may significantly underestimate the potential toxicity of NMs on various species in the environment when there is both an abundance of natural UV light which may exacerbate toxicity and NOM which may mitigate toxicity. Additionally, the formation of ecocorona's on NMs resulting from interaction with the environment has the potential to significantly change the properties of NMs and thus, alter their toxicity (Schultz et al. 2014; Noventa et al. 2018). Therefore, the most significant challenge in future nanotoxicological studies is to conduct and interpret experiments in the context of more ecologically relevant conditions, including exposing organisms to ecologically relevant concentrations for that particular organism and setting up experimental exposure conditions such as pH, IS, UV intensity and NOM concentration to match the natural conditions and variability.

References

Aich N, Plazas-Tuttle J, Lead JR, Saleh NB. Environ Chem. 2014;11:609–23. https://doi.org/10.1071/EN14127.

Apel K, Hirt H. Annu Rev Plant Biol. 2004;55:373–99. https://doi.org/10.1146/annurev.arplant.55.031903.141701.

Artells E, Issartel J, Auffan M, Borschneck D, Thill A, Tella M, Brousset L, Rose J, Bottero J-Y, Thiery A. PLoS One. 2013;8:e71260. https://doi.org/10.1371/journal.pone.0071260.

Ates M, Daniels J, Arslan Z, Farah IO. Environ Monit Assess. 2013;185:3339–48. https://doi.org/10.1007/s10661-012-2794-7.

Avramescu ML, Rasmussen PE, Chenier M, Gardner HD. Environ Sci Pollut Res. 2017;24:1553–64. https://doi.org/10.1007/s11356-016-7932-2.

Baalousha M, Manciulea A, Cumberland S, Kendall K, Lead JR. Environ Toxicol Chem. 2008;27:1875–82. https://doi.org/10.1897/07-559.1.
Baalousha M, Sikder M, Prasad A, Lead J, Merrifield R, Chandler GT. Environ Chem. 2016;13:1–3. https://doi.org/10.1071/en15142.
Bacchetta R, Santo N, Valenti I, Maggioni D, Longhi M, Tremolada P. Nanotoxicology. 2018;12:201–23. https://doi.org/10.1080/17435390.2018.1430258.
Badawy AME, Luxton TP, Silva RG, Scheckel KG, Suidan MT, Tolaymat TM. Environ Sci Technol. 2010;44:1260–6. https://doi.org/10.1021/es902240k.
Bar-Ilan O, Louis KM, Yang SP, Pedersen JA, Hamers RJ, Peterson RE, Heideman W. Nanotoxicology. 2012;6:670–9. https://doi.org/10.3109/17435390.2011.604438.
Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, Feldman GC, Milligan AJ, Falkowski PG, Letelier RM, Boss ES. Nature. 2006;444:752. https://doi.org/10.1038/nature05317.
Bian S-W, Mudunkotuwa IA, Rupasinghe T, Grassian VH. Langmuir. 2011;27:6059–68. https://doi.org/10.1021/la200570n.
Blewett TA, Leonard EM. Environ Pollut. 2017;223:311–22. https://doi.org/10.1016/j.envpol.2017.01.028.
Bour A, Mouchet F, Verneuil L, Evariste L, Silvestre J, Pinelli E, Gauthier L. Chemosphere. 2015;120:230–6. https://doi.org/10.1016/j.chemosphere.2014.07.012.
Braydich-Stolle LK, Schaeublin NM, Murdock RC, Jiang J, Biswas P, Schlager JJ, Hussain SM. J Nanopart Res. 2009;11:1361–74. https://doi.org/10.1007/s11051-008-9523-8.
Bruneau A, Turcotte P, Pilote M, Gagne F, Gagnon C. Aquat Toxicol. 2016;174:70–81. https://doi.org/10.1016/j.aquatox.2016.02.013.
Buchalska M, Kobielusz M, Matuszek A, Pacia M, Wojtyła S, Macyk W. ACS Catal. 2015;5:7424–31. https://doi.org/10.1021/acscatal.5b01562.
Bystrzejewska-Piotrowska G, Golimowski J, Urban PL. Waste Manag. 2009;29:2587–95. https://doi.org/10.1016/j.wasman.2009.04.001.
Cerrillo C, Barandika G, Igartua A, Areitioaurtena O, Mendoza G. Sci Total Environ. 2016;543:95–104. https://doi.org/10.1016/j.scitotenv.2015.10.137.
Clement L, Hurel C, Marmier N. Chemosphere. 2013;90:1083–90. https://doi.org/10.1016/j.chemosphere.2012.09.013.
Clemente Z, Castro V, Moura M, Jonsson C, Fraceto L. Aquat Toxicol. 2014;147:129–39. https://doi.org/10.1016/j.aquatox.2013.12.024.
Clift MJ, Bhattacharjee S, Brown DM, Stone V. Toxicol Lett. 2010;198:358–65. https://doi.org/10.1016/j.toxlet.2010.08.002.
Dahle JT, Arai Y. Int J Environ Res Public Health. 2015;12:1253–78. https://doi.org/10.3390/ijerph120201253.
Dahle JT, Livi K, Arai Y. Chemosphere. 2015;119:1365–71. https://doi.org/10.1016/j.chemosphere.2014.02.027.
Di Meo S, Reed TT, Venditti P, Victor VM. Oxidative Med Cell Longev. 2016;2016 https://doi.org/10.1155/2016/1245049.
Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC. Environ Toxicol Chem. 2001;20:2383–96. https://doi.org/10.1002/etc.5620201034.
Dominguez GA, Lohse SE, Torelli MD, Murphy CJ, Hamers RJ, Orr G, Klaper RD. Aquat Toxicol. 2015;162:1–9. https://doi.org/10.1016/j.aquatox.2015.02.015.
Fabrega J, Luoma SN, Tyler CR, Galloway TS, Lead JR. Environ Int. 2011;37:517–31. https://doi.org/10.1016/j.envint.2010.10.012.
Farkas J, Christian P, Gallego-Urrea JA, Roos N, Hassellöv M, Tollefsen KE, Thomas KV. Aquat Toxicol. 2011;101:117–25. https://doi.org/10.1016/j.aquatox.2010.09.010.
Felix LC, Ortega VA, Goss GG. Aquat Toxicol. 2017a;192:58–68. https://doi.org/10.1016/j.aquatox.2017.09.008.
Felix LC, Folkerts EJ, He Y, Goss GG. Environ Sci Nano. 2017b;4(3):658–69. https://doi.org/10.1039/C6EN00436A.

Feynman RP. There's plenty of room at the bottom: An invitation to enter a new field of physics. Handbook of nanoscience, engineering, and technology. 3rd ed. Boca Raton: CRC Press; 2012. p. 26–35.
Fouqueray M, Dufils B, Vollat B, Chaurand P, Botta C, Abacci K, Labille J, Rose J, Garric J. Environ Pollut. 2012;163:55–61. https://doi.org/10.1016/j.envpol.2011.11.035.
Fu PP, Xia Q, Hwang H-M, Ray PC, Yu H. J Food Drug Anal. 2014;22:64–75. https://doi.org/10.1016/j.jfda.2014.01.005.
Gallego-Urrea JA, Holmberg JP, Hassellöv M. Environ Sci Nano. 2014;1:181–9. https://doi.org/10.1039/C3EN00106G.
Ganguly P, Breen A, Pillai SC. ACS Biomater Sci Eng. 2018;4:2237–75. https://doi.org/10.1021/acsbiomaterials.8b00068.
Gao J, Youn S, Hovsepyan A, Llaneza VL, Wang Y, Bitton G, Bonzongo J-CJ. Environ Sci Technol. 2009;43:3322–8. https://doi.org/10.1021/es803315v.
Garcia-Reyero N, Thornton C, Hawkins AD, Escalon L, Kennedy AJ, Steevens JA, Willett KL. Environ Nanotechnol Monit Manage. 2015;4:58–66. https://doi.org/10.1016/j.enmm.2015.06.001.
George S, Pokhrel S, Xia T, Gilbert B, Ji Z, Schowalter M, Rosenauer A, Damoiseaux R, Bradley KA, Mädler L. ACS Nano. 2009;4:15–29. https://doi.org/10.1021/nn901503q.
Grillo R, Rosa AH, Fraceto LF. Chemosphere. 2015;119:608–19. https://doi.org/10.1016/j.chemosphere.2014.07.049.
He X, Fu P, Aker WG, Hwang H-M. J Environ Sci Health C. 2018;36:21–42. https://doi.org/10.1080/10590501.2017.1418793.
Heitmann T, Goldhammer T, Beer J, Blodau C. Glob Chang Biol. 2007;13:1771–85. https://doi.org/10.1111/j.1365-2486.2007.01382.x.
Inshakova E, Inshakov O. World market for nanomaterials: structure and trends. MATEC web of conferences. 2017;129. https://doi.org/10.1051/matecconf/201712902013.
Ispas C, Andreescu D, Patel A, Goia DV, Andreescu S, Wallace KN. Environ Sci Technol. 2009;43:6349–56. https://doi.org/10.1021/es9010543.
Iversen T-G, Skotland T, Sandvig K. Nano Today. 2011;6:176–85. https://doi.org/10.1016/j.nantod.2011.02.003.
Ježek P, Hlavatá L. Int J Biochem Cell Biol. 2005;37:2478–503. https://doi.org/10.1016/j.biocel.2005.05.013.
Ji J, Long Z, Lin D. Chem Eng J. 2011;170:525–30. https://doi.org/10.1016/j.cej.2010.11.026.
Jiang J, Oberdörster G, Biswas P. J Nanopart Res. 2009;11:77–89. https://doi.org/10.1007/s11051-008-9446-4.
Keller AA, Wang H, Zhou D, Lenihan HS, Cherr G, Cardinale BJ, Miller R, Ji Z. Environ Sci Technol. 2010;44:1962–7. https://doi.org/10.1021/es902987d.
Kim K-T, Truong L, Wehmas L, Tanguay RL. Nanotechnology. 2013;24:115101. https://doi.org/10.1088/0957-4484/24/11/115101.
Klaine SJ, Alvarez PJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR. Environ Toxicol Chem. 2008;27:1825–51. https://doi.org/10.1897/08-090.1.
Kohchi C, Inagawa H, Nishizawa T, Soma G-I. Anticancer Res. 2009;29:817–21.
Kou L, Sun J, Zhai Y, He Z. Asian J Pharm Sci. 2013;8:1–10. https://doi.org/10.1016/j.ajps.2013.07.001.
Levchuk I, Marquez JJR, Sillanpaa M. Chemosphere. 2018;192:90–104. https://doi.org/10.1016/j.chemosphere.2017.10.101.
Li S, Ma H, Wallis LK, Etterson MA, Riley B, Hoff DJ, Diamond SA. Sci Total Environ. 2016;542:324–33. https://doi.org/10.1016/j.scitotenv.2015.09.141.
Lin S, Wang X, Ji Z, Chang CH, Dong Y, Meng H, Liao Y-P, Wang M, Song T-B, Kohan S. ACS Nano. 2014;8:4450–64. https://doi.org/10.1021/nn5012754.
Lopes S, Ribeiro F, Wojnarowicz J, Łojkowski W, Jurkschat K, Crossley A, Soares AM, Loureiro S. Environ Toxicol Chem. 2014;33:190–8. https://doi.org/10.1002/etc.2413.

Ma H, Brennan A, Diamond SA. Environ Toxicol Chem. 2012;31:1621–9. https://doi.org/10.1002/etc.1858.
Ma H, Williams PL, Diamond SA. Environ Pollut. 2013;172:76–85. https://doi.org/10.1016/j.envpol.2012.08.011.
Menard A, Drobne D, Jemec A. Environ Pollut. 2011;159:677–84. https://doi.org/10.1016/j.envpol.2010.11.027.
Miller RJ, Bennett S, Keller AA, Pease S, Lenihan HS. PLoS One. 2012;7:e30321. https://doi.org/10.1371/journal.pone.0030321.
Misra SK, Dybowska A, Berhanu D, Luoma SN, Valsami-Jones E. Sci Total Environ. 2012;438:225–32. https://doi.org/10.1016/j.scitotenv.2012.08.066.
Misra SK, Nuseibeh S, Dybowska A, Berhanu D, Tetley TD, Valsami-Jones E. Nanotoxicology. 2014;8:422–32. https://doi.org/10.3109/17435390.2013.796017.
Mitrano DM, Motellier S, Clavaguera S, Nowack B. Environ Int. 2015;77:132–47. https://doi.org/10.1016/j.envint.2015.01.013.
Nason JA, McDowell SA, Callahan TW. J Environ Monit. 2012;14:1885–92. https://doi.org/10.1039/C2EM00005A.
Nasser F, Davis A, Valsami-Jones E, Lynch I. Nano. 2016;6:13. https://doi.org/10.3390/nano6120222.
Noventa S, Rowe D, Galloway T. Environ Sci Nano. 2018;5:1764–77. https://doi.org/10.1039/c8en00175h.
Oh N, Park J-H. Int J Nanomedicine. 2014;9:51. https://doi.org/10.2147/IJN.S26592.
Oi LE, Choo M-Y, Lee HV, Ong HC, Hamid SBA, Juan JC. RSC Adv. 2016;6:108741–54. https://doi.org/10.1039/C6RA22894A.
Omar FM, Aziz HA, Stoll S. Sci Total Environ. 2014;468:195–201. https://doi.org/10.1016/j.scitotenv.2013.08.044.
Orbea A, Gonzalez-Soto N, Lacave JM, Barrio I, Cajaraville MP. Comp Biochem Physiol C Toxicol Pharmacol. 2017;199:59–68. https://doi.org/10.1016/j.cbpc.2017.03.004.
Osborne OJ, Lin S, Chang CH, Ji Z, Yu X, Wang X, Lin S, Xia T, Nel AE. ACS Nano. 2015;9:9573–84. https://doi.org/10.1021/acsnano.5b04583.
Paquin PR, Santore RC, Wu KB, Kavvadas CD, Di Toro DM. Environ Sci Pol. 2000;3:175–82. https://doi.org/10.1016/S1462-9011(00)00047-2.
Paterson G, Ataria JM, Hoque ME, Burns DC, Metcalfe CD. Chemosphere. 2011;82:1002–9. https://doi.org/10.1016/j.chemosphere.2010.10.068.
Quigg A, Chin W-C, Chen C-S, Zhang S, Jiang Y, Miao A-J, Schwehr KA, Xu C, Santschi PH. ACS Sustain Chem Eng. 2013;1:686–702. https://doi.org/10.1021/sc400103x.
Quik JT, Lynch I, Van Hoecke K, Miermans CJ, De Schamphelaere KA, Janssen CR, Dawson KA, Stuart MAC, Van De Meent D. Chemosphere. 2010;81:711–5. https://doi.org/10.1016/j.chemosphere.2010.07.062.
Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Biochem J. 2004;377:159–69. https://doi.org/10.1042/bj20031253.
Ren C, Hu X, Zhou Q. NanoImpact. 2016;2:82–92. https://doi.org/10.1016/j.impact.2016.07.002.
Riley P. Int J Radiat Biol. 1994;65:27–33. https://doi.org/10.1080/09553009414550041.
Rodrigues ET, Lopes I, Pardal MÂ. Environ Int. 2013;53:18–28. https://doi.org/10.1016/j.envint.2012.12.005.
Römer I, White TA, Baalousha M, Chipman K, Viant MR, Lead JR. J Chromatogr A. 2011;1218:4226–33. https://doi.org/10.1016/j.chroma.2011.03.034.
Römer I, Gavin AJ, White TA, Merrifield RC, Chipman JK, Viant MR, Lead JR. Toxicol Lett. 2013;223:103–8. https://doi.org/10.1016/j.toxlet.2013.08.026.
Safari S, Eshraghi Dehkordy S, Kazemi M, Dehghan H, Mahaki B. Int J Photoenergy. 2015;2015 https://doi.org/10.1155/2015/504674.
Saleh NB, Aich N, Plazas-Tuttle J, Lead JR, Lowry GV. Environ Sci Nano. 2015;2:11–8. https://doi.org/10.1039/C4EN00104D.

Schultz AG, Boyle D, Chamot D, Ong KJ, Wilkinson KJ, McGeer JC, Sunahara G, Goss GG. Environ Chem. 2014;11:207–26. https://doi.org/10.1071/EN13221.
Scott DT, McKnight DM, Blunt-Harris EL, Kolesar SE, Lovley DR. Environ Sci Technol. 1998;32:2984–9. https://doi.org/10.1021/es980272q.
Scown TM, Santos EM, Johnston BD, Gaiser B, Baalousha M, Mitov S, Lead JR, Stone V, Fernandes TF, Jepson M. Toxicol Sci. 2010;115:521–34. https://doi.org/10.1093/toxsci/kfq076.
Seitz F, Rosenfeldt RR, Storm K, Metreveli G, Schaumann GE, Schulz R, Bundschuh M. Ecotoxicol Environ Saf. 2015;111:263–70. https://doi.org/10.1016/j.ecoenv.2014.09.031.
Shang L, Nienhaus K, Nienhaus GU. J Nanobiotechnol. 2014;12:5. https://doi.org/10.1186/1477 3155-12-5.
Sharma VK. J Environ Sci Health A. 2009;44:1485–95. https://doi.org/10.1080/10934520903263231.
Shaw BJ, Handy RD. Environ Int. 2011;37:1083–97. https://doi.org/10.1016/j.envint.2011.03.009.
Sikder M, Eudy E, Chandler GT, Baalousha M. Nanotoxicology. 2018;12:375–89. https://doi.org/10.1080/17435390.2018.1451568.
Silva T, Pokhrel LR, Dubey B, Tolaymat TM, Maier KJ, Liu X. Sci Total Environ. 2014;468:968–76. https://doi.org/10.1016/j.scitotenv.2013.09.006.
Sperling RA, Parak WJ. Philos Trans R Soc A Math Phys Eng Sci. 2010;368:1333–83. https://doi.org/10.1098/rsta.2009.0273.
StatNano, Database: Products, 2018. https://product.statnano.com/.
Tripathy N, Hong T-K, Ha K-T, Jeong H-S, Hahn Y-B. J Hazard Mater. 2014;270:110–7. https://doi.org/10.1016/j.jhazmat.2014.01.043.
Van Hoecke K, De Schamphelaere KA, Van der Meeren P, Smagghe G, Janssen CR. Environ Pollut. 2011;159:970–6. https://doi.org/10.1016/j.envpol.2010.12.010.
Varki A, Schauer R. Sialic acids. Essentials of Glycobiology. 2nd edition. 2009.
Westmeier D, Stauber RH, Docter D. Toxicol Appl Pharmacol. 2016;299:53–7. https://doi.org/10.1016/j.taap.2015.11.008.
Williamson CE, Zepp RG, Lucas RM, Madronich S, Austin AT, Ballaré CL, Norval M, Sulzberger B, Bais AF, McKenzie RL. Nat Clim Chang. 2014;4:434.
Wong SW, Leung PT, Djurišić A, Leung KM. Anal Bioanal Chem. 2010;396:609–18. https://doi.org/10.1007/s00216-009-3249-z.
Wormington AM, Coral J, Alloy MM, Damare CL, Mansfield CM, Klaine SJ, Bisesi JH, Roberts AP. Environ Toxicol Chem. 2016;36(6):1661–6. https://doi.org/10.1002/etc.3702.
Wyrwoll AJ, Lautenschläger P, Bach A, Hellack B, Dybowska A, Kuhlbusch TA, Hollert H, Schäffer A, Maes HM. Environ Pollut. 2016;208:859–67. https://doi.org/10.1016/j.envpol.2015.10.035.
Xia T, Zhao Y, Sager T, George S, Pokhrel S, Li N, Schoenfeld D, Meng H, Lin S, Wang X. ACS Nano. 2011;5:1223–35. https://doi.org/10.1021/nn1028482.
Yin T, Walker HW, Chen D, Yang Q. J Membr Sci. 2014;449:9–14. https://doi.org/10.1016/j.memsci.2013.08.020.
Yu L-p, Fang T, Xiong D-w, Zhu W-t, Sima X-f. J Environ Monit. 2011;13:1975–82. https://doi.org/10.1039/C1EM10197H.
Zhang Y, Chen Y, Westerhoff P, Crittenden J. Water Res. 2009;43:4249–57. https://doi.org/10.1016/j.watres.2009.06.005.
Zhang Y, Blewett TA, Val AL, Goss GG. Environ Sci Nano. 2018;5:476–86. https://doi.org/10.1039/C7EN00842B.
Zhao X, Wang S, Wu Y, You H, Lv L. Aquat Toxicol. 2013;136:49–59. https://doi.org/10.1016/j.aquatox.2013.03.019.
Zhu M, Wang H, Keller AA, Wang T, Li F. Sci Total Environ. 2014;487:375–80. https://doi.org/10.1016/j.scitotenv.2014.04.036.

Nano-enabled Consumer Products: Inventories, Release, and Exposures

S. F. Hansen, A. Mackevica, and M. S. Hull

Contents

General correspondence regarding the chapter and specific correspondence regarding the Nanotechnology Consumer Products Inventory should be directed to MS Hull
Correspondence regarding the Danish Nanodatabase should be directed to SF Hansen

S. F. Hansen (✉) · A. Mackevica
Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, Denmark
e-mail: sfha@env.dtu.dk

M. S. Hull (✉)
National Center for Earth and Environmental Nanotechnology Infrastructure (NanoEarth), Institute for Critical Technology and Applied Science (ICTAS), Virginia Tech, Blacksburg, VA, USA

NanoSafe, Inc., Blacksburg, VA, USA
e-mail: mahull@vt.edu

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_4

Abstract Given the challenges of simply defining the boundaries of what are or are not "nano" products, the concept of nano-specific consumer product inventories has arguably been one of the most important results from more than a decade of international investment in nanotechnology risk-related research, tools, and resources. Two inventories are considered especially important and are widely cited in peer-reviewed publications, grant applications, conferences and symposia, and the media. Those inventories are: (1) the Nanotechnology Consumer Products Inventory (CPI) developed in 2005 by the Woodrow Wilson International Center for Scholars' (WWICS) Project on Emerging Nanotechnologies (PEN) and (2) the Danish Nanodatabase established in 2012 by the Technical University of Denmark's Department of Environmental Engineering (DTU Environment). These inventories were intended to provide relevant information about consumer products that may contain engineered nanomaterials (ENMs) and thereby, based on precautionary principle and the potential for the release of ENMs (either intentionally or unintentionally), may pose unique risks to end users and environmental systems. This chapter informs others in this book by looking specifically at what we have learned through the process of curating inventories of nano-enabled products, particularly in the US and Europe. While additional work and resources are needed to improve these inventories, some initial trends have become evident through recent assessments of the CPI and Nanodatabase published by Vance et al. (Beilstein J Nanotechnol 6:1769–1780, 2015) and Hansen et al. (Environ Sci Nano 3:169–180, 2016), respectively. Their findings, which we must be careful to interpret as snapshots in time and subject to change as new products emerge and consumer trends vary, may provide important insights into critical questions such as (1) which products are most likely to contain nanoscale materials, (2) which nanomaterials are encountered most often in those products, (3) how likely nanomaterials are to be released from certain products and at what rates, (4) what analytical approaches and studies should be prioritized to help protect human health and the environment, and various others. Carefully and regularly curated inventories of nano-enabled consumer products may help researchers determine which product usage scenarios are likely to result in the release of ENMs and ENM/composite materials.

Keywords Consumer products · Danish Nanodatabase · Exposure · Inventory · Nanomaterial · Nanotechnology · Nanotechnology consumer products inventory · Release

Nano-enabled Consumer Products: Challenges, Inventories, and the Potential for Nanomaterial Release

The complexity of consumer products has increased dramatically in recent decades as multiple technology domains converge into a dizzying array of devices, wearables, toys, cosmetics, pharmaceuticals, and more. At the same time, these products

have become more personal and disposable than ever before. One needs to look no further than the smartphone in their hand (or at least very close by) to see this remarkable coalescence of twenty-first Century technology, personalization, and manufacturing efficiency. Packed neatly within a sleek structure designed to fit perfectly within the grasp of the average-sized human hand are miniaturized wireless communications systems, sassy digital assistants driven by artificial intelligence algorithms, lithium ion batteries, high-strength "glass" and a periodic table worth of advanced and nano-enabled materials for dissipating heat as they rapidly shuttle electrons from your finger to cyberspace. If the pen is truly mightier than the sword, then the modern smartphone should be worth at least a phalanx or two. Yet, as we marvel at the performance and convenience of modern consumer products, serious concerns have arisen around the increasingly sophisticated materials used to create these products and their potential to harm humans and the natural environment. These concerns are particularly significant for the nanoscale components of these products, which have been precisely engineered at size scales in the realm of viruses and biological machinery. We know very little, or at the very least, less than we should, about the types and quantitites of engineered nanomaterials (ENMs) that are used in many of these products. Futher, very little information exists regarding the potential release of ENMs from such products across their full life cycle – from production and use to disposal/recycling (see Fig. 1). Consequently, the extent to which humans and environmental systems may be exposed to ENMs is largely speculative and subject to change as new applications of ENMs emerge in consumer products such as personalized medicines, smart textiles, and multi-functional building materials.

Given the challenges of simply defining the boundaries of what are or are not "nano" products, the concept of nano-specific consumer product inventories has arguably been one of the most important results from more than a decade of international investment in nanotechnology risk-related research, tools, and resources. Two inventories are considered especially important and are widely cited in peer-reviewed publications, grant applications, conferences and symposia, and the media. Those inventories are: (1) the Nanotechnology Consumer Products Inventory (CPI) developed in 2005 by the Woodrow Wilson International Center for Scholars' (WWICS) Project on Emerging Nanotechnologies (PEN) and (2) the Danish Nanodatabase established in 2012 by the Technical University of Denmark's Department of Environmental Engineering (DTU Environment). These inventories were intended to provide relevant information about consumer products that may contain engineered nanomaterials (ENMs) and thereby, based on precautionary principle and the potential for the release of ENMs (either intentionally or unintentionally), may pose unique risks to end users and environmental systems.

A broad range of stakeholders from students to policy-makers, industry representatives, and researchers have cited these inventories in everything from grade-school teaching curricula to international policy documents (e.g., Michelson 2008; Healy 2009; TACD 2011; Vance et al. 2015). Such inventories fill an important need to define the extent to which consumer products are likely "nano-enabled" or not. More advanced analysis of the inventories can, in some cases, yield insights into the

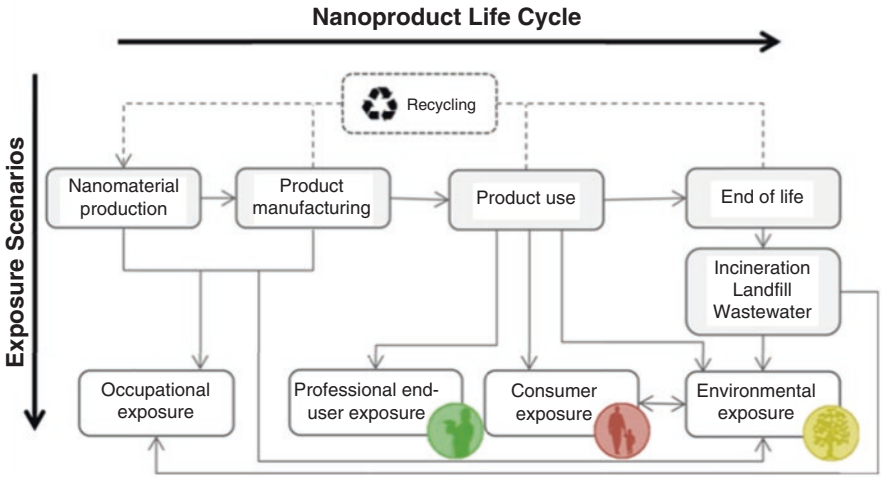


Fig. 1 Simplified stages of the nano-enabled product life cycle and the fate of the released NMs. (Mackevica 2016)

potential for the release of nanoscale materials during use or disposal of nano-enabled consumer products, and subsequent exposures to humans and the natural environment.

Other chapters in this book explore the toxicological interactions of nanomaterials with humans (e.g., chapters "Overview of Nanotoxicology in Humans and the Environment; Developments, Challenges and Impacts" and "The Potential Adverse Effects of Engineered Nanomaterial Exposure to Human Health Following Pulmonary, Oral and Dermal Exposure") and environmental systems (chapters "Overview of Nanotoxicology in Humans and the Environment; Developments, Challenges and Impacts" and "Nanotoxicology in the Environment"), methods for measuring nanoscale materials and their transformations in complex systems (chapter "Factors Affecting Nanoparticle Dose–Exposure and Cell Response"), and efforts to model exposures (chapter "Mapping Exposure onto Nanoscale Toxicity Measures"). This chapter can help inform the others by looking specifically at what we have learned through the process of curating inventories of nano-enabled products, particularly in the US and Europe. While additional work and resources are needed to improve these inventories, some initial trends have become evident through recent assessments of the CPI and Nanodatabase published by Vance et al. (2015) and Hansen et al. (2016), respectively. Their findings, which we must be careful to interpret as snapshots in time and subject to change as new products emerge and consumer trends vary, may provide important insights into critical questions such as (1) which products are most likely to contain nanoscale materials, (2) which nanomaterials are encountered most often in those products, (3) how likely nanomaterials are to be released from certain products and at what rates, (4) what analytical approaches and studies should be prioritized to help protect human health

and the environment, and various others. Carefully and regularly curated inventories of nano-enabled consumer products may help researchers determine which product usage scenarios are likely to result in the release of ENMs and ENM/composite materials.

The CPI and the Nanodatabase

A number of inventories containing information about nanomaterials and nanoproducts exist, such as the CPI, The Nanodatabase, the Nanoproduktdatenbank, maintained by the Bund für Umwelt und Naturschutz Deutschland (BUND), and the inventory established by the European Association for the Co-ordination of Consumer Representation in Standardisation (ANEC)/The European Consumer Organisation (BEUC), which contains products available on the EU market claiming to contain nanosilver particles. The CPI, which was the first of its kind, arguably tends to focus on the North American market and has only been updated about once a year since it originally launched in 2006 (PEN 2015; Vance et al. 2015). The BUND databank focuses mainly on products available on the German market and is only available in the German language, whereas the ANEC/BEUC focuses specifically on products with nanosilver and has not been updated since 2013 (BUND 2015; ANEC/BEUC 2015). Beyond Europe and North America, the Iran Nanotechnology Innovation Council has established an inventory that includes nano-enabled consumer goods, industrial products and services, nanotechnology companies, and "certified protoype" products that may be in development but are not yet commercially available (see http://nanoproduct.ir for further information).

Cosmetics regulations in the EU require that the European Commission publishes a catalogue of nanomaterials used in cosmetic products by January 2014, but the European Commission has failed to do so, supposedly due to "anomalies" in notifications by the industry (Zainzinger 2015; Hansen et al. 2016). When the catalogue was finally published in June of 2017, it only contained the name of 43 materials (e.g. alumina, cellulose, colloidal copper) and information about the category of cosmetics (e.g. face mask, nail varnish, sun protection products), exposure route (e.g. dermal) and whether the cosmetics is a rinse-off or leave on product (European Commission 2017). Brand name, nanomaterial function, particle size distribution, nanomaterial surface chemistry are among the information not included in the catalogue (Oziel 2017). Recently, the European Commission decided against the establishment of an EU-wide nanomaterial register, as it was not perceived as an appropriate way to provide information to consumers on nanomaterials, and because full coverage of all nanomaterials and mixtures would be difficult to achieve (Paun 2015). Conversely, the Belgian, Danish and French governments have proposed and established their own nanomaterial/product inventories, but any information collected so far has only been made available publically in an overview and summary format and has generally been considered not to "*...add much more to what it could be already known by an informed audience*" (BIPRO and RPA 2014).

Collectively, all of the above-mentioned inventories have a number of limitations. First of all, they are not continuously updated, meaning that months or even years may pass before the provided information is checked and revised. The lack of regular updating can lead to the listing of products that do not actually contain ENMs (false positives) as well as the exclusion of products that actually do contain ENMs (false negatives). The potential for such misrepresentation of products can erode stakeholder confidence in nano-enabled product inventories. Secondly, the inventories contain a large number of "dead" products, i.e. products that are no longer on the market. Thirdly, some of them are not available to the public, thereby preventing consumers from easy access to information regarding the products they buy. Except for The Nanodatabase, none of the inventories provide analytical tools (or in most cases, the necessary analytical data) necessary for researchers and others to perform their own independent analysis of inventoried products. And finally, the inventories do not contain any health and safety information. A comparative analysis of the different databases and inventories is provided in Table 1.

A complete review of nano-specific inventories is beyond the scope of this chapter, but consideration of the inventories and the information they do/do not contain is essential to understanding the challenges of assessing the potential for ENMs to be released from nano-enabled consumer products. Subsequent sections discuss recent findings related to the two primary inventories – the CPI and The Nanodatabase.

The Consumer Products Inventory (CPI)

The Nanotechnology Consumer Products Inventory (CPI) was developed in 2005 with support from the Woodrow Wilson International Center for Scholars (WWICS) and the Project on Emerging Nanotechnologies (PEN). The purpose of the CPI was to document the availability of "nano-enabled" products across consumer markets. The CPI is available online and freely accessible to the public at http://www.nanotechproject.org/cpi/.

The Consumer Products Inventory (CPI) launched publicly in March 2006 and offered an online tool for viewing and interacting with information related to consumer products that were claimed to contain nanoscale materials. Initially, the CPI listed about 200 such products. The total number of products now listed exceeds 1800. The CPI provided the test case through which the challenges noted earlier (namely the tedious process of updating product entry/exit from consumer markets and the limited health and safety data available) could be discovered, and various follow-on efforts have been undertaken to address them. These efforts have included modification of the original CPI (see Vance et al. 2015) and the development of new resources such as The Nanodatabase (which is discussed in greater detail in section "The Nanodatabase and Use of Nanomaterials in Consumer Products in the EU").

When the CPI launched in 2006, information on the roughly 200 products originally listed could be curated manually. As the number of products grew, however,

Table 1 Overview of the scope, update frequency, sources, limitations and strengths of different databases and inventories

Name	Est	Scope	Update frequency	Sources	Limitations	Strengths	Reference
The Nanodatabase	2012	1. Products claimed to contain NMs or be based on NT 2. Products available to European consumers	Daily	1. Online search 2. Reporting by users	1. Based on claims 2. Specifically focused on EU	1. Updated daily 2. Possible for users to do their own analysis 3. Includes hazard potential evaluation (NanoRiskCat) 4. Publically available	Hansen et al. (2016) and Aschberger et al. (2014)
CPI	2006	1. Products claimed to contain NMs or be based on NT 2. Products available globally	Annually	1. Online search 2. Reporting by users	1. Based on claims 2. Only updated periodically 3. Tends to have focus on the American market	1. Evaluation of claims in regard to credibility 2. Publically available	CPI (2015), Wijnhoven et al. (2010) and Vance et al. (2015)
ANEC/BEUC	2010	1. Products claimed to contain nanosilver 2. Products available to European consumers	Unclear	1. Online search 2. Reputing by users	Not updated since 2013	1. Publically available	ANEC/BEUC (2015) and Wijnhoven et al. (2010)
CSF Nanotechnology in Food	2015	1. Food products claimed to contain NMs	Unclear	Other nanodatabases e.g. The Nanodatabase	1. Based on other databases	1. Publically available	CSF (2015)

(continued)

Table 1 (continued)

Name	Est	Scope	Update frequency	Sources	Limitations	Strengths	Reference
BUND Nanoproduktdatenbank	2010	1. Products claimed to contain NMs or be based on NT 2. Products available in Germany	Unclear	1. Online search 2. Reporting by users	1. Only available in German 2. Tends to have focus on the German market	1. Publically available	BUND (2010, 2015) and Wijnhoven et al. (2010)
French NM compulsory reporting scheme	2013	Substance manufactured at the nanoscale	Annually	Producers, importers or distributers of at least 100 g/year	1. Limited information made publically available e.g. chemical name and uses of NMs	1. Reporting mandatory by manufacturers	Paun (2013b) and BIPRO and RPA (2014)
Belgian NM registry	2016	Substance manufactured on the nanoscale	Annually	Producers of at least 100 g/year	1. Not publically available 2. Exemptions include e.g. cosmetic products, biocides, treated products	1. Reporting mandatory by manufacturers	Paun (2013a), BIPRO and RPA (2014) and Chemical Watch (2014a)

Name	Est	Scope	Update frequency	Sources	Limitations	Strengths	Reference
Danish nanoproduct registry	2014	Nanoproducts available in Denmark	Annually	Producers and importers to report products containing or releasing nanomaterials	1. Exemptions include food contact materials, cosmetics, mixtures, printed products, textiles containing NMs in colours or dyes; paints, wood preservatives, glues and fillers, that contain nanoscale pigments used solely as colorants, rubber products that contain nano carbon black or silicon dioxide and products containing a) unintentionally produced NMs. b) "fixed" NMs 2. Information about concentration of the nanomaterial in the product, particle size distribution and specific surface area is voluntary 3. Not publically available	1. Reporting mandatory by manufacturers	Paun and Chynoweth (2014) and BIPRO and RPA (2014)

From Hansen et al. 2016

Note: Since the publication of this table in Hansen et al. (2016) a new database has been added from Iran. It can be accessed at: http://nanoproduct.ir

manual curation became a challenge that was not easily addressed. When funding for the inventory from the nonprofit Pew Charitable Trusts ended in August of 2009, product curation effectively ceased. In 2013, Virginia Tech's Institute for Critical Technology and Applied Science (ICTAS) contributed funds to support updating of the CPI and a conversion to a crowd-sourced curation mechanism. The initial results of that effort are detailed by Vance et al. (2015). No new resources have been contributed to the CPI since the Vance et al. publication and efforts to keep the CPI operational are largely voluntary and crowd-based. A key takeaway here is that databases such as the CPI are critically dependent on continuous funding. While they may offer excellent utility at one particular point in time, that utility can be diminished as new products enter consumer markets (and older ones exit) and new information about a particular product becomes available.

Throughout its existence the CPI has suffered from three primary challenges: (1) limited information, (2) limited resources, and (3) unrealistic expectations from stakeholders given challenges 1 and 2. Generally speaking, information on the composition of consumer products is remarkably limited, regardless of whether or not they are "nano-enabled". Listing of products on the CPI is based on manufacturer claims of nano-based components and the "reasonableness" of such claims by researchers. Without additional information, particularly verification by a reputable third-party, such claims can be (and indeed were) widely contested/debated. Overcoming the information gap requires sustained financial resources to help verify claims or to identify nano-enabled products in situations where claims are not made at all. The cost of such an effort is difficult to estimate, but the Virginia Tech contribution of approximately $40,000 USD helped position the CPI for crowd-based support. It remains to be determined whether or not the crowd can be motivated to adequately support the curation of the CPI amidst continued entry/exit of nano-enabled products to consumer markets. The longer challenges 1 and 2 persist, the more likely they are to erode stakeholder confidence in the CPI. From the CPI's launch it has been criticized as much for what it is not (i.e., a professionally curated database backed by rigorously vetted scientific data) nearly as often as it has been cited for what it is (i.e., an approximation of the growing number of products that are thought to contain nanocale materials and which are readily available to consumers). Looking ahead, it is unclear how the CPI can remain useful to the stakeholders it serves without regular investement into enhanced curation and verification efforts. More effective funding models for sustaining shared resources like the CPI may exist; identifying or developing such models should be prioritized for further study.

Vance et al. (2015) described the results of the Virginia Tech-led effort to update the CPI and integrate a crowd-sourcing component intended to help sustain curation of nano-enabled consumer products through the "crowd". New product categories were added to help convey the amount and type of information available for each product listed and a survey was performed to identify strategies to help meet the expectations of stakeholders who use the CPI. Key findings from Vance et al. are summarized in the sections immediately following.

The Missing Data Conundrum

One of the most notable findings from the Vance et al. study is the lack of data available on which to determine and categorize nano-enabled products. Suitable data would include, at a minimum, information such as ENM composition, concentration, and median size. More than 70% of the products listed on the CPI lack any information to support the claim that a given product indeed includes one or more ENM ingredients. Hull proposed to address this shortcoming by assigning products to one of five general categories based on the information available to support manufacturer claims. The categories, which are described in greater detail in Vance et al. (2015), were extensively verified (Category 1), verified (Category 2), manufacturer-supported (Category 3), unsupported (Category 4), and known but not claimed (Category 5). In general, these descriptors identify the nature of information provided in support of a product's claimed ENM composition. For example, the ENM composition of Category 1 products have been extensively verified through multiple peer-reviewed publications and direct observation (e.g., electron microscopy images). Only nine products (less than half a percent of the 1814 products listed at the time), could be assigned to Category 1. Most products were assigned to Category 4 (unsupported claim). Of the supporting information available to support product claims, most was provided directly by the manufacturer (Category 3). It is worrisome to note that the second largest grouping of products was Category 5, which suggests that an increasing number of manufacturers may eliminate claims of ENM composition altogether. Absent manufacturer claims and regulatory drivers to promote information exchange with stakeholders, it will be difficult to track the entry/exit of nano-enabled products moving forward.

Silver ENMs Dominate the CPI

About half of the products listed on the CPI include no information about their composition. For the products that do include this information, however, ENMs composed of silver are most frequently encountered. Not surprisingly, "antimicrobial protection", which is frequently associated with silver ENMs (but also other ENMs, such as those comprised of titanium dioxide) was most often cited as the expected benefit of incorporating ENMs into consumer products. While silver dominates the CPI in terms of number of products listed, it is unclear whether this is the case in terms of mass or volume of ENMs used in commerce. Further, it is unclear if the relatively high number of products citing silver ENM composition is the result of a reporting bias. Owing in part to the known toxicity of ionic silver, nanoscale silver became a focal point of the nanotoxicology community at an early stage relative to the greater introduction of nano-enabled consumer products. As a result, CPI curators may have been disproportionately biased to seek and list products containing silver ENMs. Thus, the potential for over-reporting of silver ENM-containing products within the CPI cannot be ruled out.

Major Product Categories

More than 40% of the products listed on the CPI are contained within the Health and Fitness category. The abundance of products in this category may, in part, reflect the relatively high use of silver ENMs to impart antimicrobial properties to certain personal care products and articles of clothing, which are two of the largest subcategories of the Health and Fitness grouping. It is worth noting that the number of products listed in the Health and Fitness category dropped by nearly 30% between 2012 and 2014 due to archiving of products that no longer met CPI listing criteria. Applications of ENMs in consumer products within the Home and Garden and Automotive sectors have increased over the last decade.

Exposures

Detailed exposure assessments for most of the products listed on the CPI are not possible due to the inherent data gaps noted previously. Despite such limitations, one may still infer likely exposure routes based on the intended use of a particular product and the general location of ENMs within that product according to previous work by Hansen et al. (2008a) (e.g., ENMs located on the surface of a product or within a liquid suspensions may be more likely to be released than ENMs located within the bulk). Inferring exposure routes in this manner for a subset of CPI-listed products, Vance et al. concluded that dermal exposures were likely to dominate due to the relatively high fraction of products characterized by ENM surface coatings or ENM-containing fluids that were meant to be touched or applied to the skin/hair. In addition to dermal exposures, ingestion or inhalation were also notable routes of potential exposure to ENMs from CPI-listed products.

Similar Findings Across Inventories

In general, the findings reported by Vance et al. for the CPI are similar to those reported by Hansen et al. (2016) following their analysis of The Nanodatabase. Both efforts cite four key findings – (1) the ENM composition of most products is unknown; (2) of the listed products with known composition, ENMs comprised of silver are most frequently disclosed; (3) the antimicrobial benefits imparted by ENMs are popular among manufacturers of nano-enabled consumer products; (4) some products no longer meet criteria for listing on either the CPI or The Nanodatabase. While the current section has focused on analysis of the CPI by Vance et al., the next section takes a closer look at The Nanodatabase and previous work by Hansen et al. (2016). Subsequent sections of the chapter will focus more heavily on The Nanodatabase as this resource has been updated more frequently and more recently than the crowd-sourced CPI.

The Nanodatabase and Use of Nanomaterials in Consumer Products in the EU

In order to address the limitations of previous inventories, The Nanodatabase (www.nanodb.dk) was established in 2012 by DTU Environment at the Technical University of Denmark, the Danish Consumer Council and the Danish Ecological Council. The Nanodatabase is an online inventory of products claimed by manufacturers or others in Europe (e.g. retailers, product reviews) to contain nanomaterials. Along with a description of the product, The Nanodatabase provides available exposure/hazard information. Moreover, to broaden its usefulness, The Nanodatabase is equipped with different analytical tools, thereby allowing the user to sort and extract data in different ways (Hansen et al. 2016). The Nanodatabase originally contained a little more than 1200 products and now has information about more than 3000 products.

Through research by Hansen et al. (2016), it was found that most of the products fall into the category of "Health and Fitness" and "Home and Garden". Personal care products and clothing are the predominant subcategories when it comes to "Health and Fitness" whereas cleaning products are by far the largest subcategory of "Home and Garden". The most used NMs are silver and titanium dioxide, but it is not possible to identify the NMs used for almost 60% of the products in the database. The following sections summarize information available in The Nanodatabase and data published by Hansen et al. (2016) and Mackevica et al. (2016a). The most up-to-date information can be found at www.nanodb.dk.

Development of Nanoproduct Commercialization

Similar to what has been observed with the CPI, the number of products contained in The Nanodatabase has increased steadily over time: 1212 products were originally in the database from the outset in 2012, and this number had risen to more than 2200 by 2015 (see Fig. 2). At the beginning of 2017, more than 3000 products can be found in The Nanodatabase. This increase in the number of products is primarily the result of increased nanoproduct marketing, as nanomaterials are employed in new applications. A total of 59 products have been retracted from the market and 16 products have lost their "nanoclaim" since 2012.

Distribution of Nanoproducts in Product Categories and Subcategories

Most of the products listed in The Nanodatabase belong to the product category "Health and Fitness" (55%), followed by "Home and Garden" (21%) and "Automotive" (12%) (see Fig. 3).

In The Nanodatabase, individual product categories include a number of subcategories, for instance personal care, clothing and cleaning (see Fig. 4). In some cases,

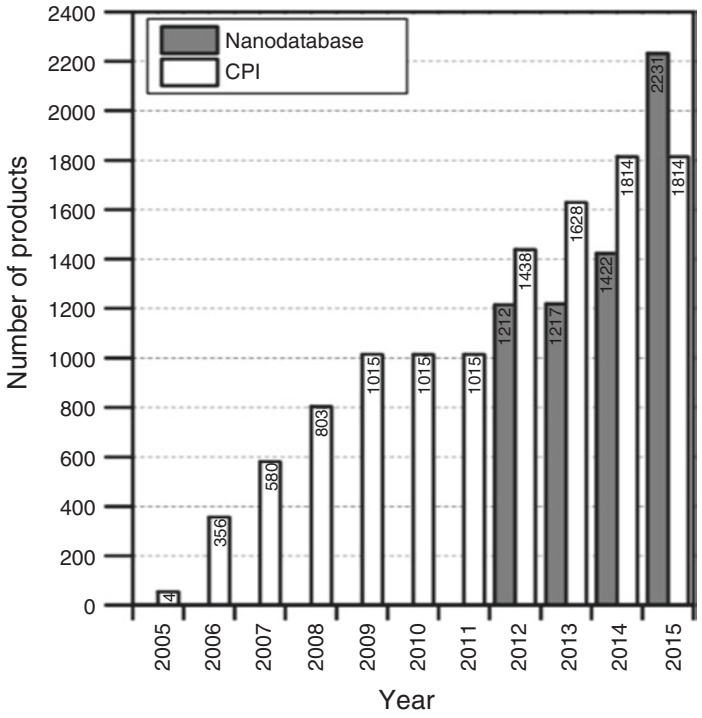


Fig. 2 Number of products listed in The Nanodatabase in the period January 2012 to August 2015 and in the Consumer Product Inventory (CPI) in the period 2005–2015. (Hansen et al. 2016)

for example in the "Health and Fitness" category, products fall into several different subcategories, suggesting a broad range of applications of nanotechnologies in a specific field (see Fig. 4a). In other cases, such as "Home and Garden", nanomaterial utilisation is restricted to fewer or single subcategories, thereby indicating potential for the further development and utilisation of nanotechnologies in this area (see Fig. 4b).

Nanomaterials Reported to Be Used

Figure 5 shows the identity of nanomaterials that are claimed to be used across the various product categories in The Nanodatabase. The analysis shows that silver is the most prominently used nanomaterial across all product categories (see Fig. 5). Other nanomaterials are specifically relevant to specific product categories: carbon nanotubes and bamboo charcoal in "Health and Fitness"; titanium dioxide in "Health and Fitness" and "Home and Garden"; gold in "Appliances", "Health and Fitness" and "Home and Garden"; titanium in "Automotive", "Health and Fitness" and "Home and Garden" and phosphate in "Appliances". Similar to the findings

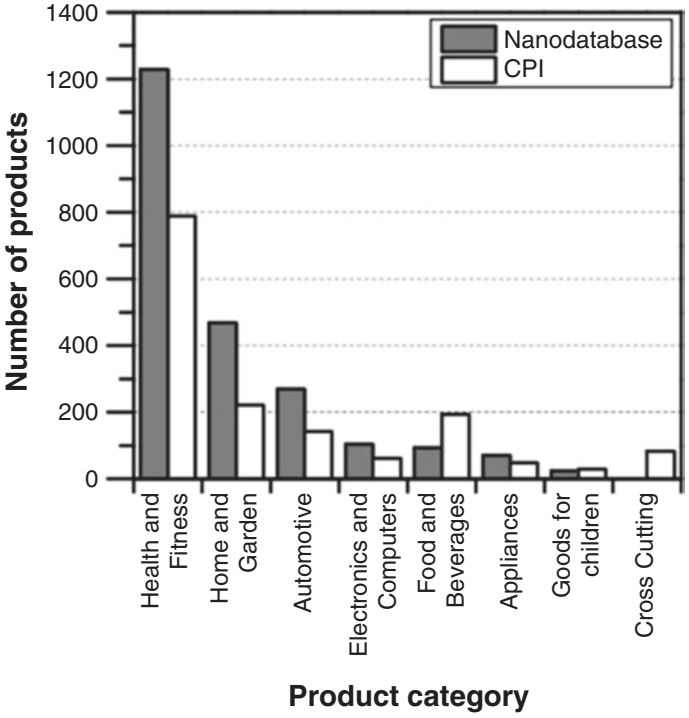


Fig. 3 Distribution of products in categories in The Nanodatabase and the CPI

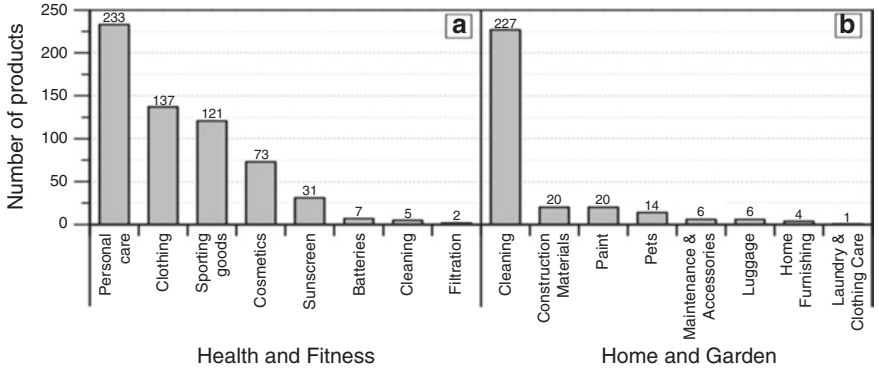


Fig. 4 Distribution of (**a**) "Health and Fitness" products in subcategories; (**b**) "Home and Garden" products in subcategories

reported by Vance et al., it should also be noted that for a large number of products it was not possible to identify and/or report the type of nanomaterial employed, due to the lack of information provided by the manufacturer. This was especially the case for the product categories "Automotive", "Electronics and Computers" and

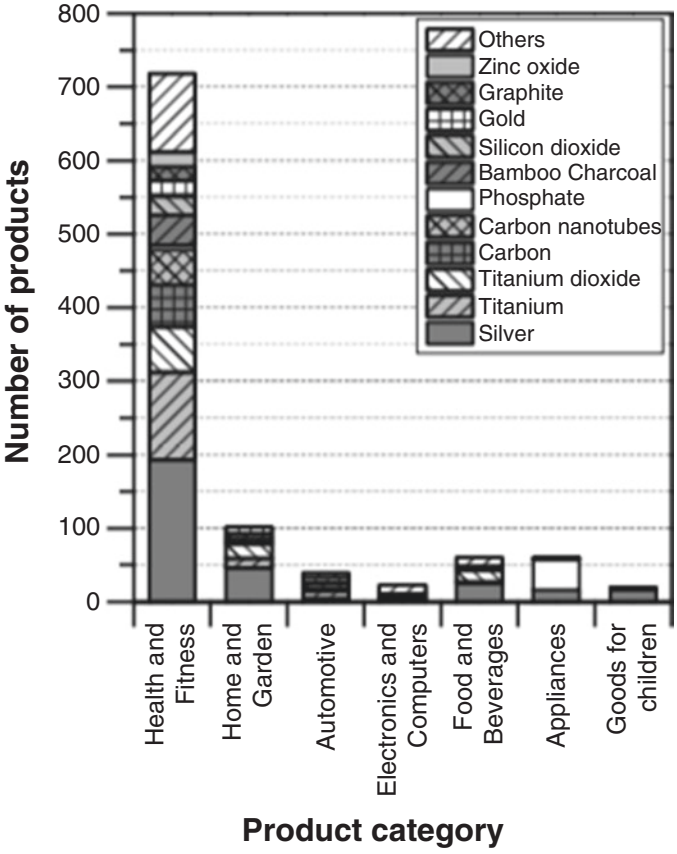


Fig. 5 Identity of nanomaterials claimed to be used in different product categories. Products where the used nanomaterial is "unknown" are excluded. Please note that individual products may have more than one type of nanomaterial. (From Hansen et al. 2016)

"Home and Garden", where 89%, 79% and 80% of the products, respectively, could not be associated with a specific nanomaterial type. The share of unknown nanomaterial was 15%, 17%, 35% and 47% for "Appliances", "Goods for Children", "Food and Beverages" and "Health and Fitness", respectively. The Nanodatabase (and CPI, the BUND Nanoproduktdatenbank and other public inventories) only contains products in which the manufacturer or others claim comprise nanomaterials, though nanomaterials are also used in consumer products where the manufacturer does not disclose this information publically. In 2012, the European Commission (2012) published a so-called Staff Working Paper (SWP) to accompany the Second Regulatory Review on Nanomaterials. From this SWP, it is clear that a wide range of nanomaterials is used in products and processes that could potentially be relevant to consumers. For instance, silica is well known to be used widely in the food industry (e.g. for clarifying wine, beer and fruit juice), but according to the data collected

in The Nanodatabase, its use is not declared in any of the more than 90 products listed in the "Food and Beverages" category as of 2016 (Hansen et al. 2016). There are two products with nanosilica in this category, but both of them are reported by third parties to contain nanoparticles. Similarly, carbon black and carbon nanotubes are used widely in the automotive industry but do not appear under that category in the database.

The lack of reporting the identity of nanomaterials is a major limitation to any effort to obtain an overview of what kind are actually being used in products available to European consumers, as well as to any kind of subsequent exposure and hazard evaluation. Knowing the identity of the nanomaterial or chemical substance is the starting point for any exposure assessment, hazard evaluation or risk assessment. It is noteworthy that even for the category "Cosmetics", in which products containing nanomaterials must be labelled with the term "[nano]" as part of the list of ingredients according to the European Cosmetics Directive, the identity of the nanomaterial is not reported for almost 50% of the items found in The Nanodatabase (Hansen et al. 2016).

Biocidal Products and Treated Articles

A number of NMs are utilised as biocides, due to their antimicrobial or antifungal properties, but little is known about to what extent biocidal products containing NMs are available on the market. The current list of approved substances, under the Biocidal Product Regulation (BPR), and those substances being examined under the Review Programme, gives a good indication as to what kinds of nanomaterials might be used in biocidal products in the EU (Mackevica et al. 2016b). This list currently contains a number of materials which are commercially available in nanoform, namely basic copper carbonate, boric oxide, copper (II) oxide and copper hydroxide (Nanowerk 2016). It is unknown whether the nanoforms of these materials are sold as biocidal ingredients in Europe, although some are clearly being marketed as such, such as the "biocidal copper carbonate nanoparticles" sold by the German company nanoSaar (Hansen and Brinch 2014; Mackevica et al. 2016b). So far, only synthetic amorphous silicon dioxide (SAS) has been approved as an active substance in the BPR as a product type (PT) 18 (insecticide). Silicon dioxide (as a nanomaterial formed by aggregates and agglomerates) and silver adsorbed on silicon dioxide (HeiQ AGS-20) are currently under review for PT 18 and PT 9 categorisations, respectively (ECHA 2016a, b). Considering the list of existing active substances that are currently under review, it is clear that at least some of them might also be available in the nanoform, for instance silver, copper, dicopper oxide and silicon dioxide. See Table 2 for substances currently being examined under the review programme which might be available in the nanoform, and the product types in which they have been notified for use.

Many NMs are used in consumer products due to their biocidal activity; for example, the antibacterial properties of nanosilver and nano-copper are exploited in various products such as antifouling paints, cleaning products, socks, toothbrushes

Table 2 Substances being examined under the Review Programme that might be available in the nanoform and the Product Types that they have been notified to be used in

	PT1	PT2	PT4	PT5	PT7	PT9	PT11	PT18	PT21
Silver		X	X	X			X		
Silver phosphate glass		X			X	X			
Silver-Zinc-Zeolite		X	X	X	X	X			
Silver copper zeolite		X	X	X	X	X			
Silver adsorbed on silicon dioxide						X			
Silver zeolite		X	X	X	X	X			
Silicium dioxide								X	
Dicopper oxide									X
Copper									X

From Mackevica et al. 2016b

and many others (Mackevica et al. 2016b). Out of the 2329 products in The Nanodatabase claimed to contain nanomaterials and estimated to be on the European market as of 2016, 342 contain nanosilver, 48 contain silicon dioxide and six contain copper (see Fig. 6).

Most of the products that use biocidal nanomaterials fall into the "Health and Fitness" (for example personal care products and clothing), "Home and Garden" (cleaning products) or "Food and Beverages" (food supplements, storage and cooking) categories. Around 100 products contain titanium dioxide, which can be considered as an active substance, though it must be noted that it is also widely used as a pigment. In about half of all the nanosilver- containing products in The Nanodatabase, the producers make antibacterial or antifungal claims.

According to an analysis carried out by Mackevica et al. (2016b), The Nanodatabase contains 88 biocidal products in total, and most of them are representing product types 1 and 2, i.e. human hygiene products and disinfectants and algaecides, respectively (Fig. 6b). Silver is the nanomaterial that is most often used as the active substances in those biocidal products (46 products), but almost half of them contain nanomaterials of unknown identity (39 products). Most of the biocidal products fall into the "home and garden" category, which is for the most part represented by different cleaning products, detergents and paints, corresponding to product type 2 – disinfectants and algaecides – according to the BPR (Fig. 3).

In total, there are 202 nano-enabled treated articles reported in The Nanodatabase as of 2016, and most of them (157) have nanosilver as the active substance (see Fig. 7a). Other nanomaterials used in treated articles include bamboo charcoal, nano iron, gold and titanium. The largest proportion of nano-enabled treated articles (79%) fall into the "Health and Fitness" category, representing different textiles, personal care items and food contact materials (Fig. 7b) (Mackevica et al. 2016b).

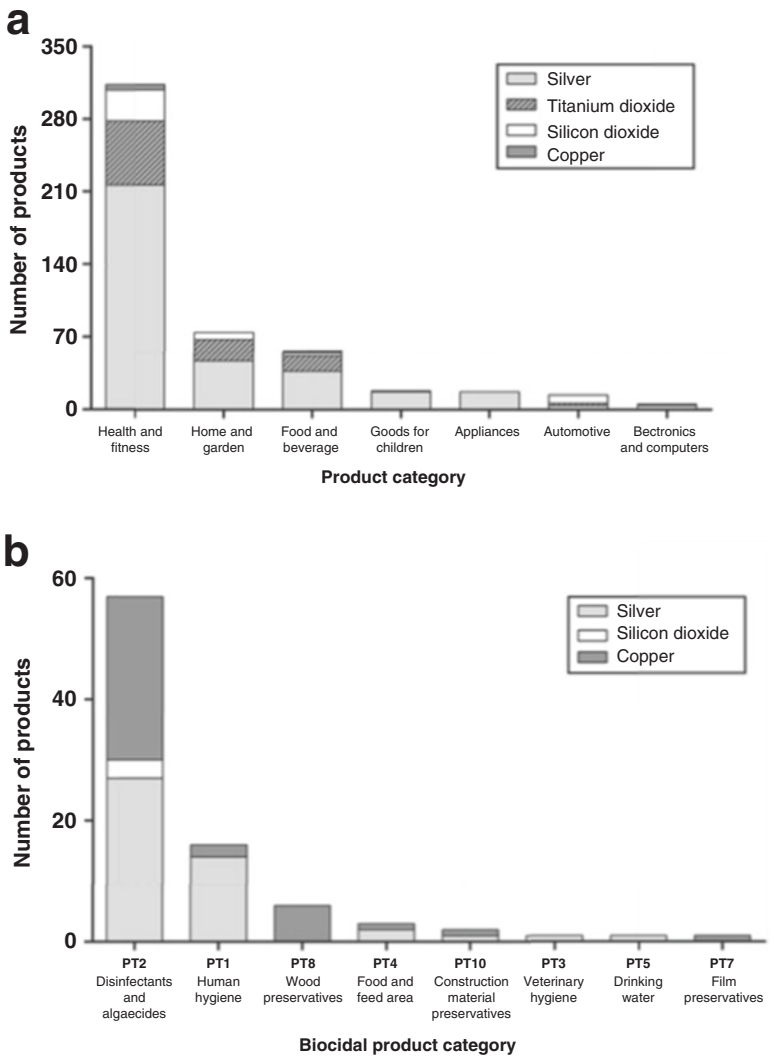


Fig. 6 Biocidal substances found in nano-enable consumer products (**a**) and the use of nano-enabled biocidal products in different biocidal product types (**b**). (From Mackevica et al. 2016b)

The Release of Nanomaterials from Consumer Products

Understanding which products do or do not contain ENMs is the first challenge. The next challenge lies in determining whether and to what extent ENMs may be released from products that do contain them. Such releases may occur either through normal use of the product or as an unintended side effect of its use.

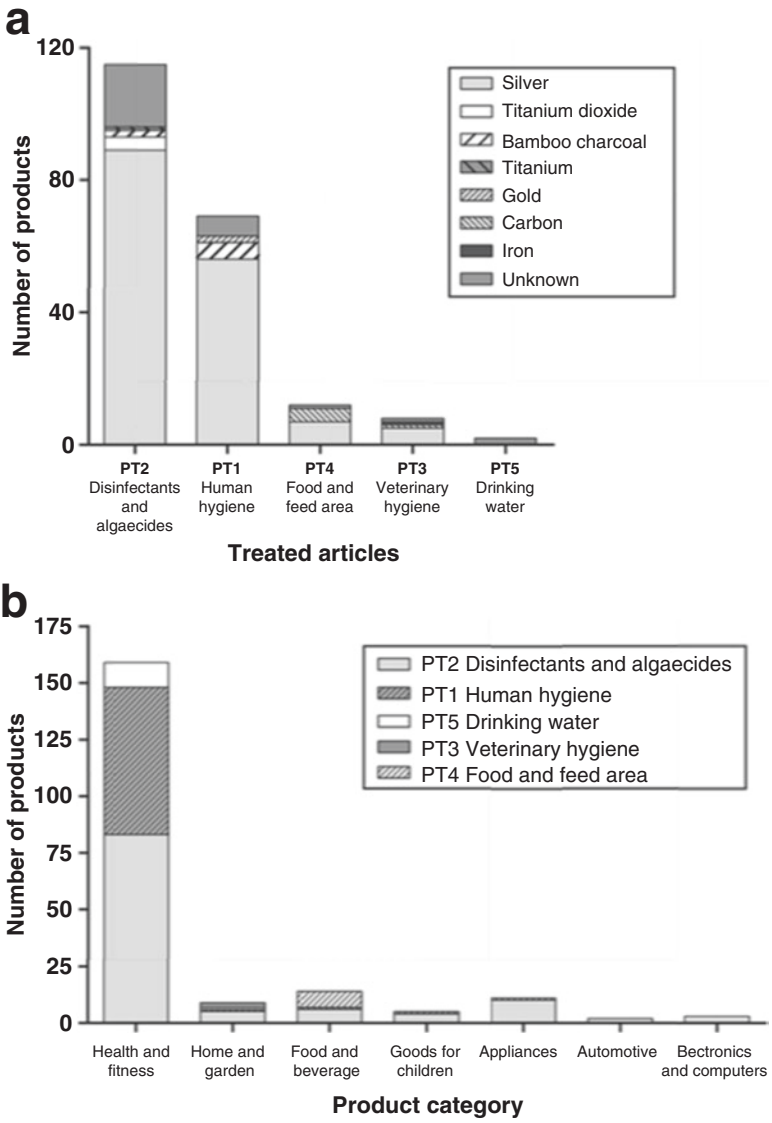


Fig. 7 Nanomaterials used in different treated articles according to the biocidal product types (**a**) and treated article product types distributed across different product categories in The Nanodatabase (**b**). (From Mackevica et al. 2016b)

In 2014, Froggett et al. (2014) published a review on the release of nanomaterials from solid nanocomposites, by identifying a total of 54 experimental studies describing nanomaterial release (Froggett et al. 2014).

A review by Koivisto et al. (2017) attempted to construct a release library based on the available experimental data on release from nanocomposites. They found 60

studies describing released fragments (airborne particles with unspecified composition), and 36 studies reporting quantitative release from various solid materials. Based on these studies Koivisto et al. were able to identify 374 different release scenarios.

A different review by Mackevica and Hansen (2016) aimed at compiling experimental studies on quantified mass release from solid nanocomposites, and investigated the extent to which information and data found in these studies could be used to perform a consumer exposure assessment according to existing REACH requirements. The numbers of scientific publications of relevance have increased substantially over the last years, and in total, 76 studies were identified as of 2014, when the review was conducted (see Fig. 8a). Most of the studies analysed the release of Ag and TiO_2 from textiles and paints, as well as CNT and SiO_2 from various nanocomposites (see Fig. 8b).

While the aforementioned 76 studies have provided some quantitative information regarding release of NPs from various items, only a handful of studies have attempted to quantify and characterize the nanomaterial present in consumer products, and only a small number of release experiments report quantitative data on released NP size and quantity. One example is a study by Mackevica et al. (2016c) on the release of total Ag and Ag NP from commercially available adult and children's toothbrushes. Using inductively coupled plasma-mass spectrometry (ICP-MS) analysis, single-particle ICP-MS and transmission electron microscopy (TEM), Mackevica et al. (2016c) found that the median size of the released Ag NPs ranged from 42 to 47 nm, and the maximum total Ag release was 10.2 ng per toothbrush, corresponding to <1% of total Ag present in the toothbrush bristles. Nano-specific release including information on particle sizes and quantitities has also been reported in numerous studies investigating AgNP migration from food contact materials (Echegoyen and Nerín 2013; von Goetz et al. 2013; Mackevica et al. 2016d), and TiO_2 release from fabrics (Wagener et al. 2016; Mackevica et al. 2018b).

When analyzing release of ENMs from products, several factors come into play, such as the nature of the tested product, the experimental setup, imagined use scenarios, different production methods employed by different producers of seemingly the same product and even the batch of the tested product (Mackevica and Hansen 2016). Because of these factors, the results can vary significantly from study to study, which is why several review articles have provided recommendations to use standardized test guidelines, harmonize data reporting and use state-of-the-art analysis methods for NP detection and characterization (Koivisto et al. 2017; Jokar et al. 2017; Mackevica and Hansen 2016).

Only a small number of studies have attempted to replicate findings from previous studies or follow standardized test guidelines (e.g. ISO guidelines for color fastness in textiles, artificial weathering for paints and varnishes, or European Commission guidelines for food contact materials), which hampers the overall ability to interpret the value of the information and data generated. However, by investigating four brands of commercially available plastic food storage containers, using European Commission test standards (Commission Regulation EU 10/2011) for plastic materials and articles intended to come into contact with food and SP-ICP-MS

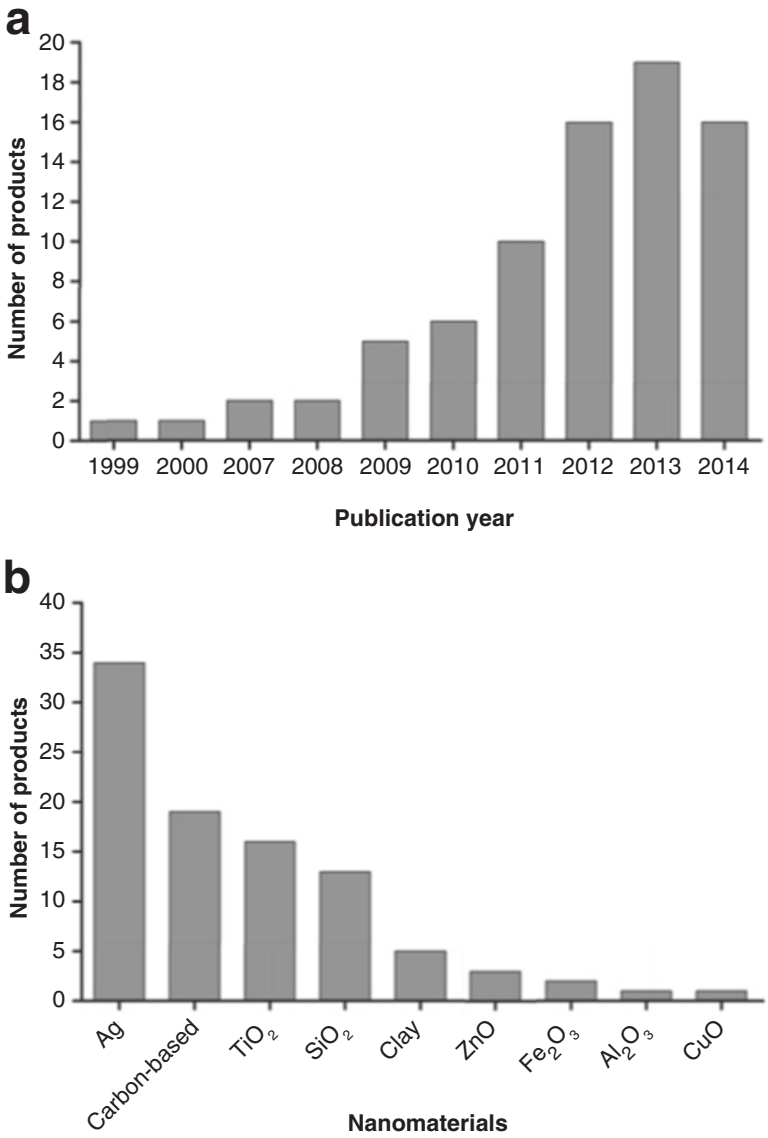


Fig. 8 Published literature on release from NM-containing solid nanocomposites grouped by year of publication (**a**) and published literature on release from NM-containing solid nanocomposites grouped by NM composition (**b**). (From Mackevica and Hansen 2016)

and TEM- EDS, Mackevica et al. (2016d) have attempted to replicate the findings of Hauri and Niece (2011) and von Goetz et al. (2013) in regard not only to Ag NP released from plastic containers and amounts leached from food containers, but also in regard to the size distribution of particulate fractions. Mackevica et al. (2016d) found that the total mass and the median size of released particulate Ag were

Table 3 Dissolved and particulate Ag leached into food simulants, measured by spICP-MS

Product	Release medium	Dissolved Ag (μg/L)	Particulate Ag (10^6 partides/L)	Paniculate Ag (ng/L)	Median size (nm)
Frcsher Longer™	MilliQ	–	37.6	13.9	30.3
Miracle Pood	10% Ethanol	–	2.8	0.5	23.9
Storage™ bags	3% Acetic acid	6.79	–	–	–
The Original	MilliQ	0.57	18.3	10.5	41.1
Always Fresh	10% Ethanol	0.66	9.5	7.1	35.5
Containers™	3% Acetic acid	10.71	2.0	27.5	89.6
Kinetic Go	MilliQ	–	2.7	0.1	17.4
Green™ Premium	10% Ethanol	0.13	7.4	2.5	26.9
	3% Acetic acid	3.18	4.2	27.8	67.2
Special Nanosilver	MilliQ	0.03	5.5	4.5	29.8
Mother's milk pack	10% Ethanol	–	5.8	1.4	25.5
	3% Acetic acid	7.51	1.9	18.3	63.8

From Mackevica et al. 2016d

generally highest in 3% acetic acid for three out of four food container brands. The total content of silver in the containers varied from 13 to 42 g/g. Similar to Hauri and Niece (2011), Mackevica et al. (2016d) found that the highest migration was observed in the 3% acetic acid food simulant for all four brands of containers, with total silver release up to 3.1 ng/cm^2 after 10 days at 40 °C (see Table 3).

Although the body of literature on the release of nanomaterials from consumer products is growing, little of the information provided in currently available studies is of relevance to regulatory exposure assessment models. In a regulatory context, both in EU (REACH) and USA (U.S. Food and Drug Administration) nano-specific exposure metrics are not required. In principle, inhalation, dermal, oral and environmental exposure estimates can be derived using REACH guidelines, but it is clear that the models and metrics are not yet developed to take into consideration the unique properties of nanomaterials. It has been acknowledged that further research is needed in order to develop more relevant consumer exposure models of nanomaterials and nanoproducts, and to develop more generalised methods for representing nanomaterial release from different product groups in relevant conditions (Larsen et al. 2015; Mackevica 2016; Mackevica and Hansen 2016). It is worth noting that similar efforts are needed to develop more effective methods for assessing environmental exposures to ENMs (Nowack 2017).

Nanomaterial Analysis in Consumer Products

Findings reported by Vance et al. (2015) and Hansen et al. (2016) for the the CPI and The Nanodatabase, respectively, indicate that there is a large number of consumer products available on the market that claim to contain ENMs. The verification of such claims can be complicated, since the manufacturers rarely describe essential information, such as the chemical composition or size of the ENM(s) or how and at what quantity it is incorporated in the product. For instance, textiles belong to one class of nano-enabled products where it is particularly difficult to determine ENM composition based solely on information provided by the manufacturer, one of the reasons being that there are numerous techniques for textile impregnation with ENMs. For example, a particular ENM could be either incorporated within the bulk of the textile fibers, or simply added to surface of the fibers, or woven into the fabric as threads. Without knowing the identify and properties of the ENM used, as well as where it is located and how it was manufactured, one must apply rigorous experimental/analytical investigations to arrive at a reasonable determination of this information. For instance, according to The Nanodatabase, out of the 129 products that are produced in Denmark, 122 are listed as "unknown" when it comes to ENM type, which makes it close to impossible to verify whether or not the product actually contains ENM, and what is the chemical identity of those ENMs.

Further complicating these efforts is the fact that the exact methods of ENM integration into many materials may be highly proprietary to industrial producers of nano-enabled products. For example, an analysis of the linkages between US patents and prior research demonstrated that "papers in nanoscience and nanotechnology, materials science, and biomaterials were the most closely linked to patents" (Ahmadpoor and Jones 2017). With so much economic incentive understandably limiting what producers disclose about their nano-enabled products and processes, it is likely that the challenges of determining the precise ENM composition of products and industrial process will persist for years if not decades to come. Even with strong regulatory drivers, policy-makers would likely need to carefully limit disclosure requirements to ensure that enabling intellectual property is protected.

Analytical Methods and Sample Preparation

There are various methods available that allow either direct characterization of ENMs in the product or require extraction of particles from their product matrix to verify that ENMs are actually present in the product, and provide quantification and characterization of these ENMs. Which methods are most appropriate to use for a particular situation are highly dependent on both the type of ENM used in the product and the product matrix. Here, we focus solely on analysis of ENMs comprised of metal and metal oxides, which are among the most popular NMs in consumer products. We will also limit our discussion to methods that are useful for

determining the physico-chemical identity of the ENM as there there are many analytical techniques available for quantifying the size of ENMs irrespective of composition. These techniques include, for example, Dynamic Light Scattering (DLS) or Nanoparticle Tracking Analysis (NTA). Chapter "Factors Affecting Nanoparticle Dose–Exposure and Cell Response" of this book includes additional discussion of nano-metrology techniques, and an overview of state-of-the-art analytical methods together with their analytical capabilities and size detection limits can be viewed in a recent literature review (Laborda et al. 2016).

As noted above, suitable analytical methods and sample preparation procedures can be selected depending on what matrix the product represents – whether it is a spray (e.g. disinfectants and cleaning products), liquid (e.g. dietary supplements, personal care products, paints), or solid (e.g. sports equipment, food contact materials, textiles) (see Fig. 9).

Analytical methods that are applicable for ENM analysis in consumer products can be separated into two general categories – (1) quantitative and (2) qualitative. Quantitative methods provide information on ENM chemical identity, size and concentration, and are based primarily on spectrometry techniques. Such methods include inductively coupled plasma – mass spectrometry (ICP-MS), which can be used in single particle mode ICP-MS (SP-ICP-MS), or coupled with pre-separation steps, such as field-flow fractionation (FFF). These analyses require that samples be in a liquid form, so appropriate sample preparation steps are necessary for ENMs in complex matrices. Solid articles may be subjected to various extraction procedures (e.g., acid, alkaline or solvent extraction, enzymatic digestion) as well to liberate the ENMs from the matrix. Alternatively, if the product is in a powder form, less complicated sample preparation methods might be sufficient, such as dispersion in water or other suitable medium. Products that are already in liquid form may be directly analyzed after dilution or pre-concentration, or will require phase separation or ENM extraction steps before analysis, depending on the composition of the liquid. Each sample type presents different characteristics that have to be taken into consideration for choosing an appropriate method for sample preparation and analysis. For instance, products like cosmetics and sunscreens have a high fat content, and will require appropriate sample preparation steps for NM analysis (Nischwitz et al. 2012). Other samples, such as water-based dietary supplements or liquid food products may be directly analyzed, or diluted and analyzed (Qu et al. 2014; Peters et al. 2014). For analysis of airborne samples, ICP-MS can be coupled with a Scanning Mobility Particle Sizer (SMPS), which is applicable for various water-based spray products (Losert et al. 2015).

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) are usually used as qualitative ENM characterization methods, but can in principle be used to obtain quantitative information. Coupled with Energy-Dispersive X-ray Spectrometry (EDX), electron microscopy can provide information on particle size, shape, and chemical composition. If possible, it is usually recommended to complement the results from spectrometry methods with microscopy investigations, and vice versa (Mackevica and Hansen 2016). Usually, for electron microscopy analysis, the sample is fixed over a solid support as a thin film.

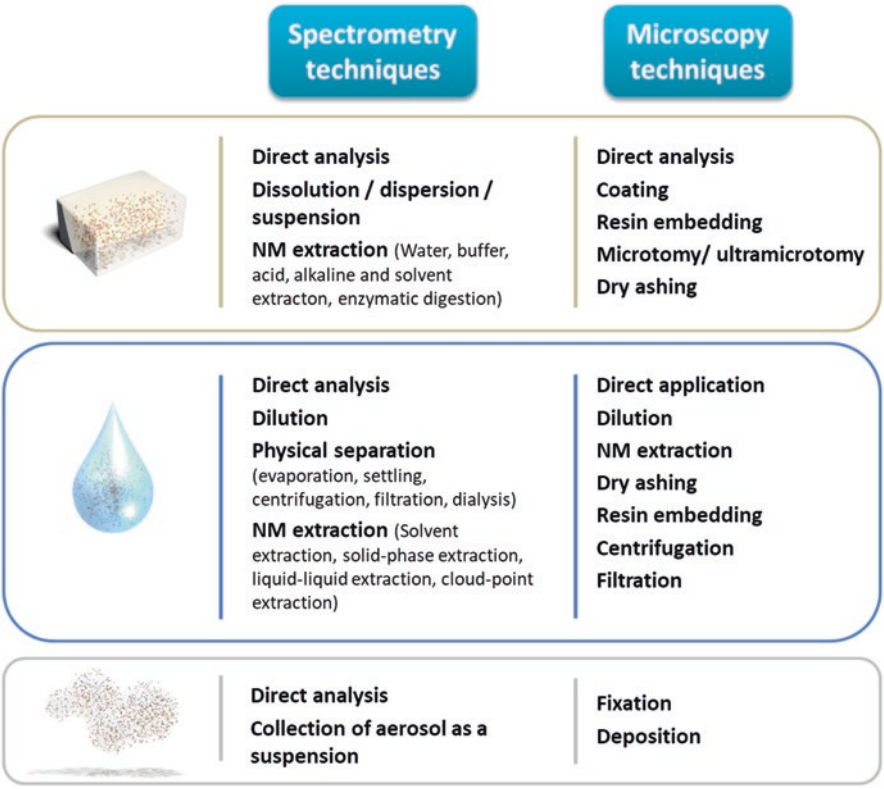


Fig. 9 Overview of main sample preparation procedures for solid, liquid and gaseous samples according to the analytical technique. Based on the following literature reviews – De La Calle et al. (2016), Laborda et al. (2016), and Mackevica and Hansen (2016)

To achieve this, various sample preparation procedures are necessary, which can be cumbersome and time consuming. The sample cannot usually be analyzed as an aqueous suspension (with few exceptions that require special instruments), so the main procedures involve depositing and drying the sample, and subsequently coating it with a conductive layer if necessary. Analysis of some solid samples may require cross-sectioning to facilitate examination of ENMs embedded in the matrix or coated on the surface. Preparation of cross-sections is very time-consuming, and consists of embedding the sample in epoxy resin prior to thin sectioning by cryo-ultramicrotomy (De La Calle et al. 2016). This sample preparation method requires special training and availability of expensive equipment, such as a diamond knife for thin-sectioning of the sample. A simpler approach to finding ENMs in complex matrices is dry ashing (heating the sample in a muffle at high temperatures), which has been applied for sample preparation of various textiles (Benn and Westerhoff 2008; Kulthong et al. 2010), food storage boxes (Huang et al. 2011), and sunscreens

(Dan et al. 2015). However, this method involves destroying the sample matrix and may introduce artefacts.

Generally speaking, even when it comes to analyzing ENMs in consumer products comprised of challenging matrices, a variety of techniques are available to facilitate thorough characterization and quantification of the ENMs present. However, oftentimes the process can be lengthy, cumbersome and costly, as many samples may require extensive sample preparation for ENM extraction, which can result in particle transformations, such as dissolution, aggregation, changes in particle size, lack of sample stability and representativity, formation of salts (chlorides or sulphides) and matrix complexity, which subsequently includes higher level of uncertainty during sample analysis. Therefore, the process of choosing appropriate and compatible sampling methods and analytical techniques is critical to obtaining reliable data on the ENM composition of nano-enabled consumer products.

Several examples of ENM analysis in consumer products and sample preparation procedures are listed in Table 4 together with references to scientific studies that have performed such investigations. We have selected examples that can realistically be applied to various commercial products that are available in Denmark and the EU according to www.nanodb.dk.

Human Exposure to Nano-enabled Consumer Products

Ultimately, the primary purpose of nano-specific consumer product inventories is to better understand the scope of human and environmental exposures to ENMs and the potential for subsequent adverse health effects. The sections that follow discuss recent findings related to such exposures.

The route of exposure associated with the use of a particular nano-enabled product is essential to understanding its potential health and safety impacts. Based on the nature and intended of the product, Hansen et al. found that dermal exposure is the most prominent route of exposure for most product categories (Fig. 10). Inhalation exposure may be significant for the “Automotive” and “Home and Garden” categories, whereas, as expected, oral exposure may be more significant when considering product categories such as “Food and Beverages” and “Health and Fitness”. When looking at Fig. 10, it is important to note that the figure displays only the potential route of exposure across the individual product categories (if exposure takes place) but does not include any considerations regarding whether the exposure is high, medium or low.

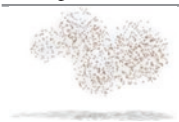
There are many products in The Nanodatabase for which the identity of the nanomaterial is not reported. For nanoproducts in the database for which nanomaterials are reported, silver is the most prominent type when it comes to dermal exposure (see Figs. 6 and 7), followed by titanium dioxide and bamboo charcoal. For inhalation, silver is also the most prevalent followed by titanium, titanium dioxide and gold. Finally, a total 34 products can lead to the oral exposure of nanosilver, whereas

Table 4 Examples of analysis of NMs in consumer products (based on the literature) with price estimates and examples of products from www.nanodb.dk for which a similar approach may be applied

Nanoproducts	Analytical methods	Sample preparation	Examples from experimental studies
 **Solids** • Plastic food containers • Fabrics • Food items • Coatings (dried) • Paints (dried)	SEM-EDX TEM-EDX	Dry ashing	• **Ag in plastic food storage boxes** (Huang et al. 2011) • **Ag in textiles** (Benn and Westerhoff 2008; Kulthong et al. 2010)
	TEM-EDX	Resin embedding and cryo-ultramicrotomy	• **Ag in plastic food storage boxes** (Addo Ntim et al. 2015) • **TiO_2 in chewing gum** (Chen et al. 2013)
	SEM-EDX	Resin embedding and polishing	• **TiO_2 in textiles** (Windler et al. 2012)
	SEM-EDX	Direct investigation	• **ZnO and Ag in fabrics** (Li et al. 2014) • **Ag in silicone keyboard covers** (Mackevica et al. 2018a) • **CuO in paint** (Mackevica et al. 2018a)
 **Liquids** • Suncreens • Personal care products • Spray products • Coatings • Paints • Dirt repellants • Beverages • Dietary supplements	SP-ICP-MS	Direct analysis, dispersion in water, solvent or surfactant	• **TiO_2 in sunscreens** (Dan et al. 2015)
	FFF-ICP-MS	Direct analysis, dispersion in water, solvent or surfactant	• **Ag in dietary supplements and antiseptic products** (Bolea and Castillo 2011) • **TiO_2 in sunscreens** (Nischwitz and Goenaga-Infante 2012)
	TEM-EDX SEM-EDX	Direct deposition, dispersion in water, solvent or surfactant	• **Paints with SiO_2, Ag, TiO_2** (Fiorentino et al. 2015) • **TiO_2, ZnO in sunscreens** (Lewicka et al. 2011) • **Ag in spray products** (Lorenz et al. 2011)
	TEM-EDX	Resin embedding and cryo-ultramicrotomy	• **Metal oxides in sunscreens** (Butler et al. 2012)
	TEM-EDX SEM-EDX	Centrifugation onto grid/ substrate	• **Ag in spray products** (Hagendorfer et al. 2010)

(continued)

Table 4 (continued)

Nanoproducts	Analytical methods	Sample preparation	Examples from experimental studies
 **Airborne** • Spray products • Coatings • Disinfectants • Personal care products	SMPS-ICP-MS	Spraying in a spray chamber	• **Ag in spray products** (Losert et al. 2015)
	SP-ICP-MS	Cooling spray cans in liquid nitrogen, opening cans and evaporating the solvent, dilution	• **Ag in spray products** (Losert et al. 2015)
	TEM-EDX SEM-EDX	Cooling spray cans in liquid nitrogen, opening cans and evaporating the solvent, dilution, and centrifugation onto grids	• **Ag in spray products** (Losert et al. 2015)
	TEM-EDX SEM-EDX	Precipitation of spray onto a grid/substrate	• **Ag in antiodor spray, throat spray, and surface disinfectant** (Quadros and Marr 2011)

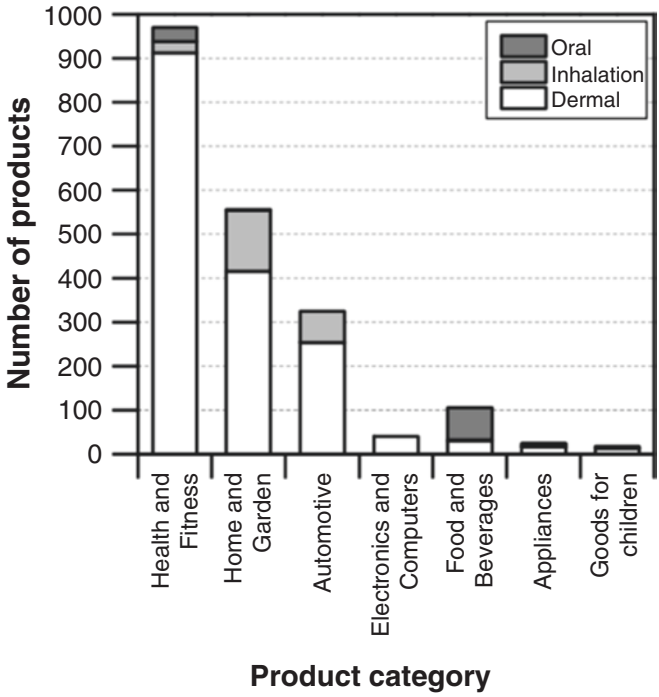


Fig. 10 Potential route of exposure for individual product categories. Please note that individual products may have more than one route of exposure. (From Hansen et al. 2016)

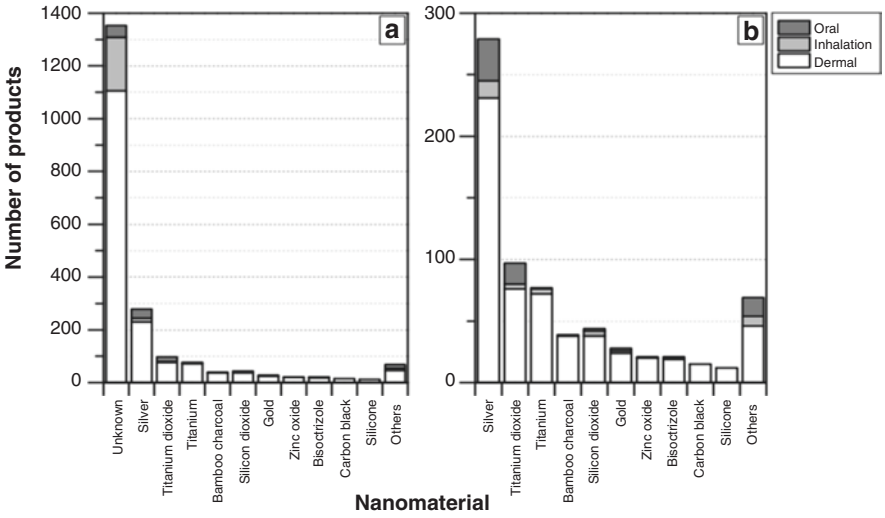


Fig. 11 Potential routes of exposure with respect to individual nanomaterials (**a**) including unknown and (**b**) excluding products where the used nanomaterial is "unknown". (From Hansen et al. 2016)

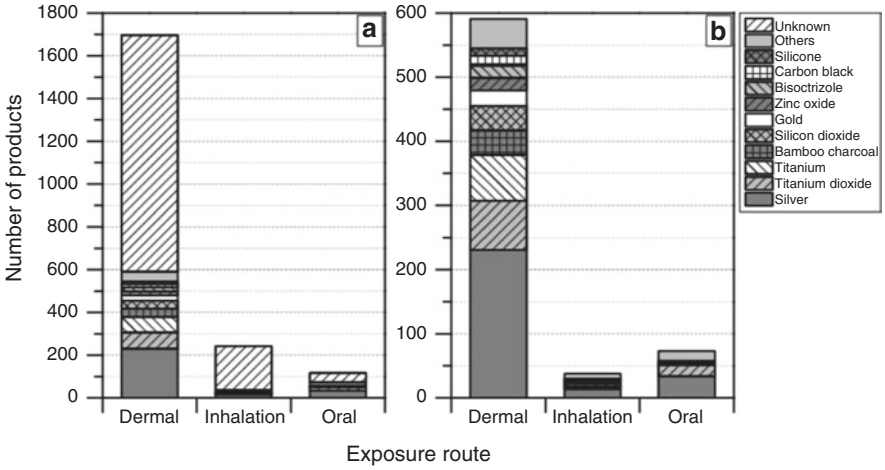


Fig. 12 Identity of nanomaterials reported according to their potential route of exposure: (**a**) including products for which the nanomaterial used is unknown and (**b**) excluding products for which the used nanomaterial is unknown. (From Hansen et al. 2016)

17 and 4 products lead to oral exposure of nanotitanium dioxide and nanocalcium, respectively (see Figs. 11 and 12).

When considering the body parts that might be exposed during use of the nano-products in The Nanodatabase, it is clear that the palm only, the face and scalp (chin, cheeks, hair) and the upper torso (hips, back, trunk, chest, loins) are the areas of the body that might be most exposed (see Fig. 13) (The Nanodatabase 2017).

Environmental Release

Historically, the environmental concentration of chemical substances has been found to increase with their use in society. These increases can be related to the intentional release of substances through their normal and routine application (e.g., agricultural fertilizers or household disinfectant sprays) or as an unintended consequence of their use in another form or product (e.g., pharmaceuticals or fuel additives). Consequently we can expect that over time, the use of nano-enabled consumer products is likely to result in increasing concentrations of ENMs in surface waters, air, groundwater and soils (Ganzleben and Hansen 2012a). As the conentrations of ENMs in the environment increase, so too does the likelihood that they may pose risks to human health and the environment.

The environmental release of ENMs may occur at different stages during the life cycle of a material (e.g. production, use and end-of-life), and can occur via multiple pathways and from multiple sources. The diversity of ENMs produced and commercialised coupled with the diversity of nano-enabled products that incorporate them may have a magnifying affect on the number and complexity of ENM release pathways. Potential point sources of ENM emissions include spills (e.g., during manufacturing, integration, or transport), industrial emissions (e.g., to air, water, soils), emissions into the air (e.g., from use at construction sites and incineration plants), effluents (released into surface waters from urban wastewater treatment plants), landfill leachates (into soil and groundwater), and direct releases of ENMs

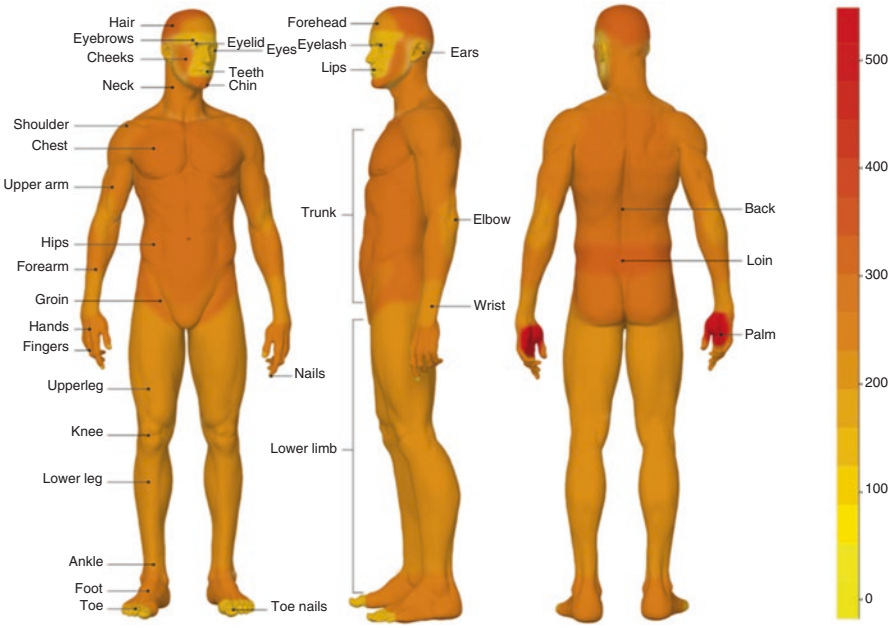


Fig. 13 Number of products in The Nanodatabase distributed across human body parts

(e.g., into soils and groundwater for remediation purposes). Diffuse sources include ENM release from products during use and re-use, ENM leaching into groundwater and then into surface waters from landfills, the run-off from agricultural land of pesticides that contain ENMs, and from sewage sludge and spilt lubricants that are washed off roads into stormwater discharges (Baun et al. 2009; Ganzleben and Hansen 2012a, b).

Available data on point-source emissions remain very limited, while a reliable estimate of diffuse source emissions from nanoproducts is currently hampered by the lack of information and lack of access to information about: volumes of ENMs on the market; volume fractions incorporated into products; market penetration and use patterns and emissions of ENM from products throughout the life cycle (Ganzleben and Hansen 2012a, b). Once in the environment, the behaviour of ENMs will depend on their physicochemical properties, and on the environment into which they are released. The fact that ENMs behave differently relative to dissolved chemicals limits the applicability of existing exposure models (Ganzleben and Hansen 2012b; Gottschalk et al. 2015). Insights into the environmental fate and pathways of ENMs has increased in the last decade to the extent that aquatic reactions of ENMs, such as dissolution and aggregation, can be modelled in complex media, especially in the case of data-rich ENM materials such as Ag (e.g. Quik et al. 2011; Dale et al. 2013). The first attempts to group different ENMs in regard to environmental fate and behavioural properties have been made, such as by Hartmann et al. (2014) (see Table 5).

Attempts have also been made to model the environmental fate and pathways of ENMs (see Ganzleben and Hansen (2012a) and Gottschalk et al. (2015) for a review; see also Nowack (2017) for a more recent review of all currently available nano fate models), suggesting a number of data characteristics relevant to environmental exposure data for ENM, including:

- Mass concentrations in the range of µg/L – pg/L depending on the environmental media and changes in concentrations over time;
- Particle properties such as size and shape and range of particle distribution, i.e. identifying and measuring the size fractions of different nanoforms;
- Available ENM surface area;
- Distinguishing between ENMs and naturally occurring nanomaterials, and;
- Data on the degree of aggregation and dissolution, i.e. ongoing fate and behaviour (Ganzleben and Hansen 2012a).

However, there are gaps in our knowledge when it comes to understanding the environmental fate and behaviour of nanomaterials. A number of these process require further study including, for example, chemical/photochemical transformation, dissolution/precipitation/speciation, agglomeration/aggregation, biological transformation, sedimentation, adsorption and desorption, and, above all, validation of appropriate characterisation and measurements for ENMs in environmentally relevant media (Hartmann et al. 2014).

Table 5 Relative importance of transformation processes for modelling the environmental fate of uncoated, non-functionalised forms of selected NMs

	Process	Importance of the environmental process in fate modelling					
		Low	*Medium*				*High*
(Photo) chemical	*Photochemical*	nZVI, CB	ZnO, CuO	Ag, CeO_2	TiO_2, CNT		
	Redox	TiO_2, CNT, CeI_2,CB	ZnO, CuO				Ag, nZVI
	Dissolution	TiO_2, CNT, nZVI, CB	CeO_2			CuO	Ag, ZnO
Physical	*Aggregation / Agglomeration*					Ag, ZnO	TiO, CNT, CuO, nZVI, CeO_2, CB.
	Sedimentation					Ag, ZnO	TiO_2, CNT, CuO, nZVI, CeO_2, CB
Interaction with surface/ substances	*NOM adsorption*					Ag, TiO_2, ZnO, CuO, nZVI, CeO_2	CNT, CB
	Sorption onto other surfaces/ retention in soil				Ag, ZnO, CuO	TiO_2, CeO_2	CNT, nZVI, CB
Biologically mediaed	*Biodegradation*	Ag, TiO_2, ZnO, CuO, nZVI, CeO_2, CB	CNT				
	Bio-modification		Ag, TiO_2, ZnO, CuO, nZVI, CeO_2, CB	CNT			

From Hartmann et al. 2014

Solid Waste Flows from Nano-enabled Consumer Products

The increasing use of ENMs in society, and specifically in consumer products, means that ENMs will eventually find their way into various forms of waste treatment processes (incineration, wastewater treatment plants, etc.), some of which

may not have been originally designed to treat such materials (Heggelund et al. 2016; OECD 2016). Very few experimental studies have investigated the fate and behaviour of pristine nanomaterials in simulated landfill conditions (e.g. Bolyard et al. 2013) and during incineration (Walser et al. 2012). Recently, Salieri et al. (2018) published a review discussing the current status of life cycle assessment (LCA) of manufactured nanomaterials.

In order to gain a better understanding of the end-of-life waste treatment of nano-enabled consumer products, Heggelund et al. (2016) used The Nanodatabase to provide an overview of ENMs flowing into and throughout waste systems in Europe, including in Denmark and the United Kingdom. First, the available nano-enabled products were categorised into waste material fractions. Then the types of ENMs present in waste material fractions were estimated, followed by an estimation of the region-specific waste management of individual waste material fractions. Finally, the information obtained was combined to determine the distribution of ENMs routed to specific waste treatment options (Heggelund et al. 2016). The largest of a total of nine different waste fractions identified by Heggelund et al. (2016) was found to be "Plastic, packaging", "Textile" and "Electronics", with 847, 390 and 306 products, respectively, out of a total of 2312 products in The Nanodatabase. The most abundant ENM across all waste fractions was found to be silver, but otherwise the second-most abundant ENM was found to vary between different waste fractions (see Fig. 14). Plastic packaging waste comprised the largest variety of ENMs, namely 20 different ENMs, which might be caused by the fact that this waste material fraction is generated from many different sources (product categories) such as the automotive, food & beverage and home & garden sectors.

By combining information on the distribution of ENM types in waste fractions with information on how the individual waste fractions are treated within the European Union (EU), Denmark (DK) and the United Kingdom (UK), Heggelund et al. (2016) estimated the relative distribution of nanoproducts to waste treatment technologies and found that more than 50% of the nanoproducts are likely to end up in recycling processes for all three regions within the nine waste fractions identified (see Fig. 15). Europe and the UK offer quite comparable incineration and landfilling treatment options, routing 19% and 13% to incineration and 26% and 29% to landfilling, respectively. Denmark, on the other hand, to a large extent, combines incineration with energy recovery, which results in 38% of nanoproducts ending up in waste incineration plants and only 8% in landfills.

By combining the distribution of ENM types in waste fractions (Fig. 14) and the relative distribution of end-of-life (EOL) nanoproducts into waste treatment options in the EU, Denmark and the UK (Fig. 15), Heggelund et al. (2016) finally derived the distribution of nanomaterials for the four different waste management options: incineration, recycling, landfilling and composting/anaerobic digestion (see Fig. 16).

From Fig. 16, one can see that 31% of EOL nano-enabled consumer products in Europe entering a waste incineration plant will contain nanosilver and that anaerobic digestion/compost is expected to be relevant for a few nanoproducts only. The distribution of NMs in the different waste management systems was found to be

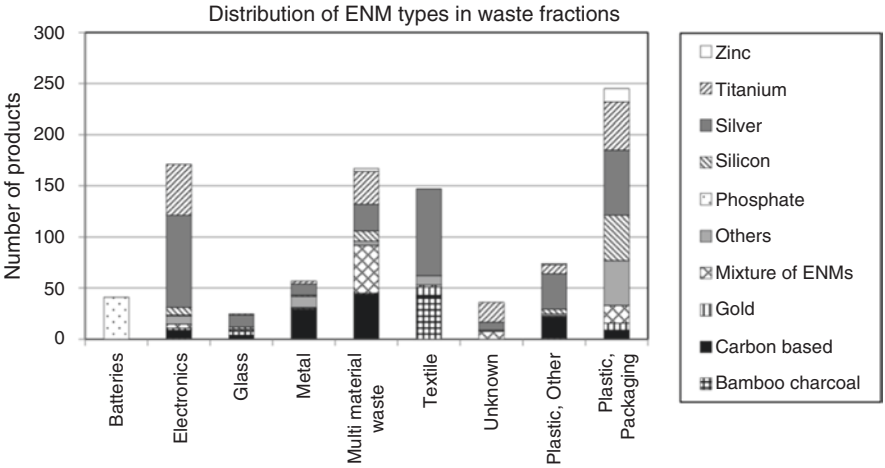


Fig. 14 Distribution of ENM in the different waste material fractions according to data from nanodb.dk. The Y-axis represents the number of products containing a certain ENM (nanodb.dk). Please note that the products have been grouped according to which primary nanotechnology substance they contain, e.g. "Titanium" includes both titanium and titanium dioxide, and "carbon based" includes CNTs, carbon black, fullerenes and graphite. (From Heggelund et al. 2016)

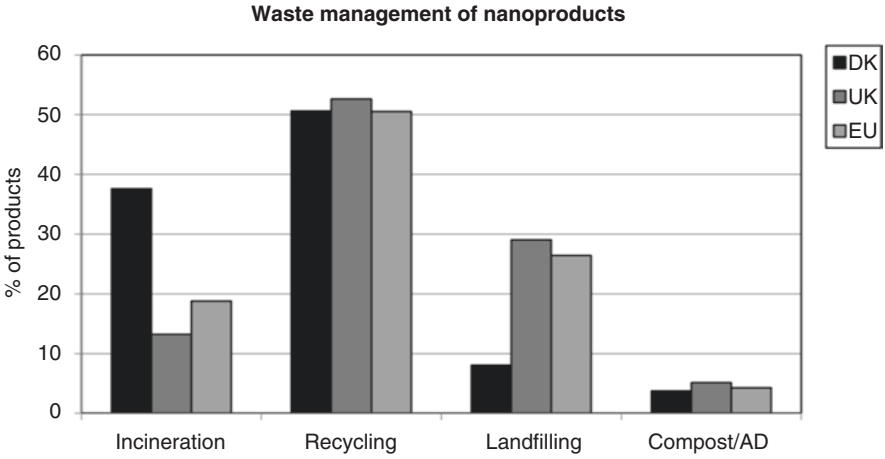


Fig. 15 Relative distribution (%) of end-of-life nanoproducts into waste treatment options in the three analysed scenarios: Europe (EU), Denmark (DK) and the United Kingdom (UK). (From Heggelund et al. 2016)

similar for Europe, e.g. the numbers of items containing silver and titanium NM were more or less the same, regardless of the management scenario. Some interesting regional differences were furthermore observed; the proportions of titanium- and carbon-based NMs were found to be higher in the UK landfill scenario, because greater amounts of plastic waste (both packaging and other plastic) are disposed of

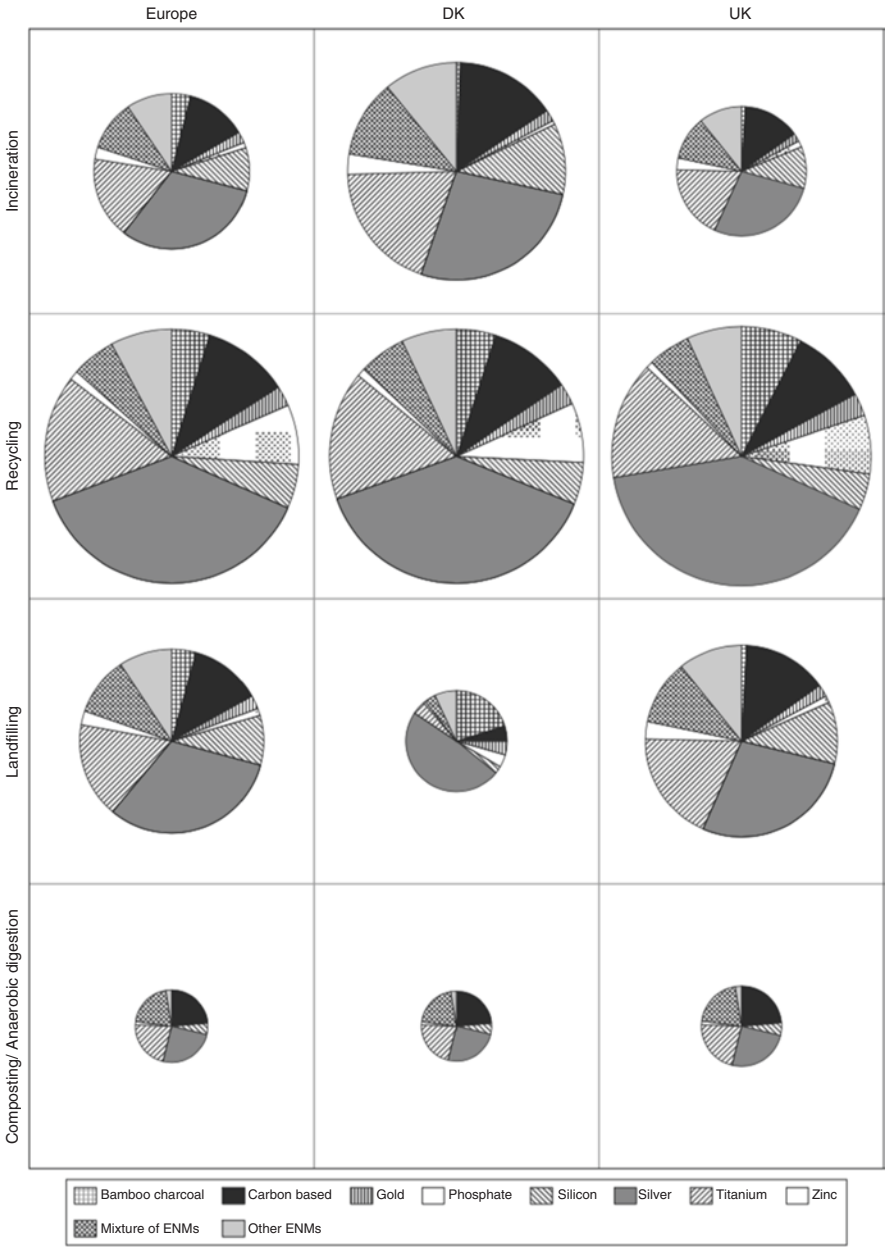


Fig. 16 Distribution of nanomaterials for the four different waste management options: incineration, recycling, landfilling and composting/anaerobic digestion. The figure illustrates the percentage of products entering a waste treatment option that will contain a certain ENM. Note: the area of the pie is proportional to the number of products entering individual treatments, thus reflecting the size of the bars in Fig. 15. (From Heggelund et al. 2016)

in landfills in the UK compared to Denmark, whereas bamboo charcoal and nano-gold are expected to be present in Danish landfills, due to the larger amounts of textile waste.

In order to assess the environmental exposure of nanoparticles from solid waste, Boldrin et al. (2014) proposed a five-step framework (see Fig. 17) and applied it to three different examples: nanosilver in polyester textiles, nano-scale titaniumdioxide in sunscreen lotion and carbon nanotubes in tennis racquets. Boldrin et al. (2014) found that considerable amounts of these nanoproducts entered waste management systems, based on data available in 2011 (globally 23.7×10^3 Mg of polyester textiles, 715–1430 Mg of sunscreen lotion and 313–825 Mg tennis racquets). On a global scale, this would result in 0.8–5.6 Mg of nanosilver, 14–143 Mg of nanoTiO_2 and 0.5–1.2 Mg of CNTs being released annually into the environment, based on potential waste management practices and exposure routes (Boldrin et al. 2014).

Boldrin et al. (2014) observed that the main challenges in relation to further research into nanomaterials and waste were: (1) the transformation of nanomaterials within waste treatment technologies, (2) release mechanisms in conditions relevant for waste disposal, (3) exposure assessments performed at the local level and within a precise context, (4) the characterisation of nanowaste and the development of appropriate analytical methods and (5) a definition of appropriate regulatory limit values and nanowaste data reporting.

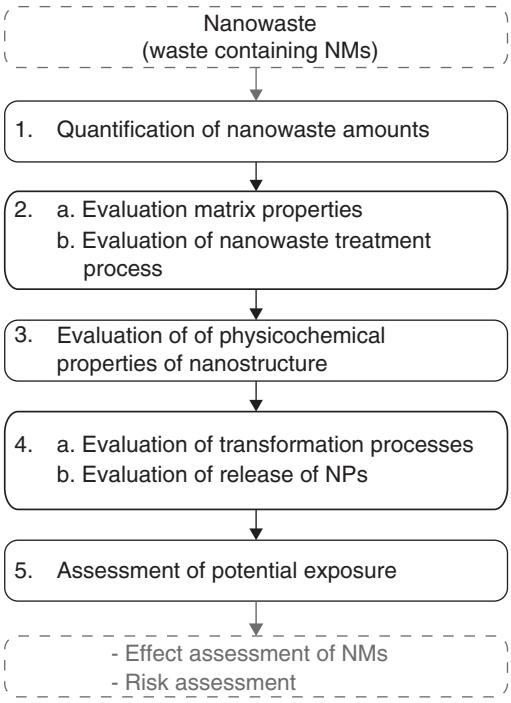


Fig. 17 Proposed framework for an environmental exposure assessment of nanoparticles in solid waste. The framework includes steps 1–5. When combined with results from an effect assessment, the results of the exposure assessment may be used as an input into the environmental risk assessment of nanoparticle emissions from waste (lower dotted box, outside the scope of the present chapter)

Conclusion and Outlook: Toxicological Implications of Exposure to Nano-enabled Consumer Products

The first step to assessing the release of ENMs from consumer products is to define which products are likely to contain them. Over the last decade, two inventories have emerged that aim to help address a wide variety of concerns related to nano-enabled consumer products, particularly the potential for nanomaterial release. Based on assessments of these inventories by multiple research groups, several key trends have emerged. First, the data provided by manufacturers are simply inadequate to assess whether or not given products do or do not contain ENMs or how likely a given product is to release ENMs during routine use or otherwise. Secondly, the products with known composition represent relatively simplistic applications of nanotechnology – i.e., first- to second-generation nano-enabled products with enhanced antimicrobial efficacy, dispersion properties, or mechanical strength. If development trends proceed as anticipated, future nanoproducts are likely to have increased complexity and properties that go beyond simply antimicrobial efficacy or increased surface area.

Investments in nanometrology and nanotechnology environmental health and safety research over the last decade have equipped researchers with the tools and protocols needed to effectively measure the ENM composition of products as well as their release into complex biological and environmental systems. The effectiveness of those tools can be greatly enhanced when manufacturers provide sufficient information about the ENM composition of their products. Ideally, manufacturers would provide this information proactively so that products entering commerce may be prioritized for risk assessment based on their ENM composition and release profile. Ultimately, such efforts could allow for the timely identification and mitigation of potentially adverse impacts to human health and the environment resulting from the release of ENMs from nano-enabled products. However, as noted previously, the maintenance of inventories like the CPI and the Nanodatabase is costly and requires sustained investment. As long as the adverse impacts of ENM are speculative or limited to controlled experiments, then securing such investments will likely remain a challenge. Conversely, should the use of ENMs in commerce lead to clear evidence of adverse impacts on humans or the environment, then regulatory actions might require manufacturers to comply with detailed reporting requirements for the ENM composition of their products Until then, such resources are likely to be maintained primarily through assemblages of concerned stakeholders, academic groups, and proactive consumer goods manufacturers.

References

Addo Ntim S, Thomas TA, Begley TH, Noonan GO. Characterisation and potential migration of silver nanoparticles from commercially available polymeric food contact materials. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2015;32:1003–11.

Ahmadpoor M, Jones B. The dual frontier: patented inventions and prior scientific advance. Science. 2017;357(6351):583–7.
ANEC/BEUC. List of nanotech. 2015. http://www.beuc.eu/publications/2013-00141-01-e.xls. Accessed 13 Aug 2015.
Aschberger K, Rauscher H, Crutzen H, Rasmussen K, Christensen FM, Sokull-Klüttgen B, Stamm H. Considerations on information needs for nanomaterials in consumer products. European Commission Joint Research Centre Institute for Health and Consumer Protection. CPI 2015. Consumer Product Inventory. Project of Emerging Nanotechnologies. 2014. Available: http://www.nanotechproject.org/cpi/. Accessed 13 Aug 2015.
Baun A, Hartmann NB, Grieger KD, Hansen SF. Setting the limits for engineered nanoparticles in European surface waters. J Environ Monit. 2009;11:1774–81.
Benn TM, Westerhoff P. Nanoparticle silver released into water from commercially available sock fabrics. Environ Sci Technol. 2008;42:4133–9.
BIPRO and RPA. Study to assess the impact of possible legislation to increase transparency on nanomaterials on the market. Brussels: DG Enterprise and Industry, European Commission; 2014.
Boldrin A, Hansen SF, Baun A, Hartmann N, Astrup TF. Environmental exposure assessment framework for nanoparticles in solid waste. J Nanopart Res. 2014;16:2394. https://doi.org/10.1007/s11051-014-2394-2.
Bolea E, Castillo JR. Size characterization and quantification of silver nanoparticles by asymmetric flow field-flow fractionation coupled with inductively coupled plasma mass spectrometry. Anal Bioanal Chem. 2011;401:2723–32.
Bolyard SC, Reinhart DR, Santra S. Behavior of engineered nanoparticles in landfill leachate. Environ Sci Technol. 2013;47(15):8114–22.
BUND. Nanoproduktdatenbank. 2015. Available: http://www.bund.net/nc/themen_und_projekte/nanotechnologie/nanoprodukt datenbank/produktsuche/. Accessed 13 Aug 2015.
BUND. BUND veröffentlicht Datenbank mit über 200 Nano-Produkten. 2010. Available: https://www.bund.net/nc/presse/pressemitteilungen/detail/zurueck/pressemitteilungen/artikel/bund-veroeffentlichtdatenbank-mit-ueber-200-nano-produkten/. Accessed: 30 Oct 2015.
Butler MK, Prow TW, Guo YN, Lin LL, Webb RI, Martin DJ. High-pressure freezing/freeze substitution and transmission electron microscopy for characterization of metal oxide nanoparticles within sunscreens. Nanomedicine [Internet]. 2012;7:541–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22394188
Chen XX, Cheng B, Yang YX, Cao A, Liu JH, Du LJ, Liu Y, Zhao Y, Wang H. Characterization and preliminary toxicity assay of nano-titanium dioxide additive in sugar-coated chewing gum. Small. 2013;9:1765–74.
Chemical Watch 2014a. Belgium to implement nanomaterials register in 2016. Chemical Watch 12 February 2014.
CSF Nanotechnology in Food. Center for Food Safety. 2015. Available: http://salsa3.salsalabs.com/o/1881/p/salsa/web/common/public/content?content_item_KEY=14112. Accessed 30 Oct 2015.
Dale AL, Lowry GV, Casman EA. Modeling nanosilver transformations in freshwater sediments. Environ Sci Technol. 2013;47(22):12920–8.
Dan Y, Shi H, Stephan C, Liang X. Rapid analysis of titanium dioxide nanoparticles in sunscreens using single particle inductively coupled plasma-mass spectrometry. Microchem J. 2015;122:119–26.
De La Calle I, Menta M, Séby F. Current trends and challenges in sample preparation for metallic nanoparticles analysis in daily products and environmental samples: A review Spectrochimica Acta Part B. Spectrochim Acta Part B [Internet]. 2016 [cited 2017 Jul 19]; 125:66–96. Available from: https://doi.org/10.1016/j.sab.2016.09.007
ECHA. Silicon dioxide (as a nanomaterial formed by aggregates and agglomerates). 2016a. Available: http://dissemination.echa.europa.eu/Biocides/factsheet?id=1449-18. Accessed 14 Mar 2016 (Accessed 02 Dec 2016).

ECHA. Silver adsorbed on silicon. 2016b. http://dissemination.echa.europa.eu/Biocides/factsheet?id=1448-09. Accessed 02 Dec 2016.
Echegoyen Y, Nerín C. Nanoparticle release from nano-silver antimicrobial food containers. Food Chem Toxicol. 2013;62:16–22.
European Commission. Commission Staff Working Paper Types and uses of nanomaterials, including safety aspects accompanying the Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee on the Second Regulatory Review on Nanomaterials SWD(2012) 288 final. Brussels: European Commission; 2012.
European Commission. Catalogue of nanomaterials used in cosmetic products placed on the EU market Version 1 (31.12.2016). 2017. Available: http://ec.europa.eu/docsroom/documents/24521. 05 Aug 2017.
Fiorentino B, Golanski L, Guiot A, Damlencourt JF, Boutry D. Influence of paints formulations on nanoparticles release during their life cycle. J Nanopart Res. 2015;17
Froggett SJ, Clancy SF, Boverhof DR, Canady RA. A review and perspective of existing research on the release of nanomaterials from solid nanocomposites. Part Fibre Toxicol. 2014;11:17. https://doi.org/10.1186/1743-8977-11-17.
Ganzleben C, Hansen SF. Environmental exposure to nanomaterials – data scoping study. Service contract no.07.0307/2011/610874/ETU/D.3. Brussels: Milieu; 2012a.
Ganzleben C, Hansen SF. Nanomaterials as priority substances under the water framework directive. Eur J Law Technol. 2012b;2:38–45.
Gottschalk F, Nowack B, Lassen C, Kjølholt J, Christensen F. Nanomaterials in the Danish environment. Modelling exposure of the Danish environment to selected nanomaterials. Environmental project no. 1639, 2015. Copenhagen: Danish Environmental Protection Agency; 2015.
Hagendorfer H, Lorenz C, Kaegi R, Sinnet B, Gehrig R, Goetz NV, Scheringer M, Ludwig C, Ulrich A. Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles. J Nanopart Res [Internet]. 2010 [cited 2016 Jul 13];12:2481–94. Available from: http://link.springer.com/10.1007/s11051-009-9816-6
Hansen SF, Brinch A. The biocides market for nano actives. Chem Watch. 2014;67:8–9.
Hansen SF, Heggelund L, Mackevica A. Nanoproducts: what is actually available to European consumers? Environ Sci Nano. 2016;3:169–80.
Hansen SF, Michelson E, Kamper A, Borling P, Stuer-Lauridsen F, Baun A. Categorization framework to aid exposure assessment of nanomaterials in consumer products. Ecotoxicology. 2008a;17:438–447.
Hartmann NB, Skjolding LM, Hansen SF, Kjølholt J, Gottschalck F, Baun A. Environmental fate and behaviour of nanomaterials new knowledge on important transformation processes environmental project no. 1594, 2014. Copenhagen: The Danish Environmental Protection Agency; 2014.
Hauri JF, Niece BK. Leaching of silver from silver-impregnated food storage containers. J Chem Educ. 2011;88:1407–9.
Healy N. Why nano education? J Nano Educ. 2009;1(1):6–7.
Heggelund L, Hansen SF, Astrup TF, Boldrin A. Semi-quantitative analysis of solid waste flows from nano-enabled consumer products in Europe, Denmark and the United Kingdom – abundance, distribution and management. Waste Manag. 2016;56:584–92.
Huang Y, Chen S, Bing X, Gao C, Wang T, Yuan B. Nanosilver migrated into food-simulating solutions from commercially available food fresh containers. Packag Technol Sci [Internet]. 2011 [cited 2016 Jul 21];24:291–7. Available from: http://doi.wiley.com/10.1002/pts.938
Jokar M, Pedersen GA, Loeschner K. Six open questions about the migration of engineered nano-objects from polymer-based food-contact materials: a review. Food Addit Contam Part A. 2017;34(3):434–50.

Koivisto AJ, Jensen ACØ, Kling KI, Nørgaard A, Brinch A, Christensen F, Jensen KA. Quantitative material releases from products and articles containing manufactured nanomaterials: towards a release library. NanoImpact. 2017;5:119–32.

Kulthong K, Srisung S, Boonpavanitchakul K, Kangwansupamonkon W, Maniratanachote R. Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. Part Fibre Toxicol [Internet]. 2010 [cited 2016 Sep 4];7:8. Available from: http://particleandfibretoxicology.biomedcentral.com/articles/10.1186/1743-8977-7-8

Laborda F, Bolea E, Cepriá G, Gómez MT, Jiménez MS, Pérez-Arantegui J, Castillo JR. Detection, characterization and quantification of inorganic engineered nanomaterials: a review of techniques and methodological approaches for the analysis of complex samples. Anal Chim Acta. 2016;904:10–32.

Larsen PB, Christensen F, Keld C, Jensen A, Brinch A, Mikkelsen SH. Exposure assessment of nanomaterials in consumer products. Environmental project no. 1636. Copenhagen: Danish Environmental Protection Agency; 2015.

Lewicka ZA, Benedetto AF, Benoit DN, Yu WW, Fortner JD, Colvin VL. The structure, composition, and dimensions of TiO2 and ZnO nanomaterials in commercial sunscreens. J Nanopart Res [Internet]. 2011 [cited 2016 Jul 13];13:3607–17. Available from: http://link.springer.com/10.1007/s11051-011-0438-4

Li Z, Tang H, Yuan W, Song W, Niu Y, Yan L, Yu M, Dai M, Feng S, Wang M, et al. Ag nanoparticle-ZnO nanowire hybrid nanostructures as enhanced and robust antimicrobial textiles via a green chemical approach. Nanotechnology. 2014;25

Lorenz C, Hagendorfer H, von Goetz N, Kaegi R, Gehrig R, Ulrich A, Scheringer M, Hungerbühler K. Nanosized aerosols from consumer sprays: experimental analysis and exposure modeling for four commercial products. J Nanopart Res [Internet]. 2011 [cited 2016 Jul 13];13:3377–91. Available from: http://link.springer.com/10.1007/s11051-011-0256-8

Losert S, Hess A, Ilari G, von Goetz N, Hungerbuehler K. Online characterization of nano-aerosols released by commercial spray products using SMPS–ICPMS coupling. J Nanopart Res [Internet]. 2015;17:293. Available from: http://link.springer.com/10.1007/s11051-015-3078-2

Mackevica, A. Release of nanomaterials from consumer products and implications for consumer exposure assessment. PhD Thesis October 2016. Kgs. Lyngby: DTU Environment Department of Environmental Engineering Technical University of Denmark; 2016.

Mackevica A, Hansen SF. Release of nanomaterials from solid nanocomposites and consumer exposure assessment – a forward-looking review. Nanotoxicology. 2016;10

Mackevica A, Revilla P, Brinch A, Hansen SF. Current uses of nanomaterials in biocidal products and treated articles in the EU. Environ Sci Nano. 2016a;3(5):1195–205.

Mackevica A, Besora PR, Brinch A, Hansen SF. Current uses of nanomaterials in biocidal products and treated articles in the EU. Environ Sci Nano. 2016b;3:1195–205.

Mackevica A, Olsson ME, Hansen SF. The release of silver nanoparticles from commercial toothbrushes. J Hazard Mater. 2016c;322(Part A):270–5.

Mackevica A, Olsson ME, Hansen SF. Silver nanoparticle release from commercially available plastic food containers into food simulants. J Nanopart Res. 2016d;18(1):1–11.

Mackevica A, Olsson ME, Mines PD, Heggelund LR, Hansen SF. Dermal transfer quantification of nanoparticles from nano-enabled surfaces. NanoImpact. 2018a;11:109–18.

Mackevica A, Olsson ME, Hansen SF. Quantitative characterization of TiO_2 nanoparticle release from textiles by conventional and single particle ICP-MS. J Nanopart Res. 2018b;20:6.

Michelson ES. Globalization at the nano frontier: the future of nanotechnology policy in the United States, China, and India. Technol Soc. 2008;30:405–10.

Nanowerk. Nanomaterials database. 2016. http://www.nanowerk.com/nanomaterial-database.php. Accessed 02 Dec 2016.

Nischwitz V, Goenaga-Infante H. Improved sample preparation and quality control for the characterisation of titanium dioxide nanoparticles in sunscreens using flow field flow fractionation on-line with inductively coupled plasma mass spectrometry. J Anal At Spectrom [Internet]. 2012 [cited 2016 Jul 13];27:1084. Available from: http://xlink.rsc.org/?DOI=c2ja10387g

Nischwitz V, Goenaga-Infante H, Bolea E, Castillo JR, Scherrers R, Ludwig C, Ulrich A, Rose J, Bottero J-Y, Zazueta C, et al.. Improved sample preparation and quality control for the characterisation of titanium dioxide nanoparticles in sunscreens using flow field flow fractionation on-line with inductively coupled plasma mass spectrometry. J Anal At Spectrom [Internet]. 2012 [cited 2017 Aug 30];27:1084. Available from: http://xlink.rsc.org/?DOI=c2ja10387g

Nowack B. Evaluation of environmental exposure models for engineered nanomaterials in a regulatory context. NanoImpact. 2017;8:38–47.

OECD. Nanomaterials in waste streams: current knowledge on risks and impacts. Paris: Organisation for Economic Co-operation and Development; 2016. Available online: http://www.oecd-ilibrary.org/environment/nanomaterials-in-waste-streams_9789264249752-en. Last accessed 23 May 2016.

Oziel C. ClientEarth files complaint over EU cosmetics nano inventory. Chem Watch. 2017. Available: https://chemicalwatch.com/58020/clientearth-files-complaint-over-eu-cosmetics-nano-inventory. Accessed 07 Aug 2017.

Paun, C.. EU nanomaterials register looks unlikely not a good way to provide information to consumers. 2015. EU Commission says. 11 December 2014. https://chemicalwatch.com/22241/eu-nanomaterials-register-looks-unlikely?q=nano%2C%20unlikely. Accessed 30 Oct 2015.

PEN Updates. Project of emerging nanotechnologies. 2015. Available: http://www.nanotechproject.org/cpi/about/updates/. Accessed 30 Oct 2015.

Peters RJ, Van Bemmel G, Herrera-Rivera Z, Helsper HPFG, Marvin HJP, Weigel S, Tromp PC, Oomen AG, Rietveld AG, Bouwmeester H. Characterization of titanium dioxide nanoparticles in food products: Analytical methods to define nanoparticles. J Agric Food Chem [Internet]. 2014 [cited 2016 May 19];62:6285–93. Available from: http://pubs.acs.org/doi/abs/10.1021/jf5011885

Paun C. Belgium notifies EU Commission of nano register plan. Chemical Watch 10 July 2013. 2013a. Available: https://chemicalwatch-com.globalproxy.cvt.dk/15632/belgium-notifies-eu-commission-ofnanoregister-plan?q=Belgium%20notifies%20EU%20Commission%20of%20nano%20register%20plan. Accessed 30 Oct 2015.

Paun C. French nanomaterials register receives 3,400 declarations. Chemical Watch 12 December 2013. 2013b. https://chemicalwatch-com.globalproxy.cvt.dk/17530/french-nanomaterials-register-receives-3400-declarations?q=French%20register

Paun, C., Chynoweth, E. 2014. Denmark launches consumer product register for nano. Chemical Watch 26 June 2014. Available: https://chemicalwatch-com.globalproxy.cvt.dk/20265/denmark-launchesconsumerproduct-register-fornano?q=Belgium%20notifies%20EU%20Commission%20of%20nano%20register%20plan; https://www.retsinformation.dk/Forms/R0710.aspx?id=163367 (Accessed 30-10-2015).

Qu H, Mudalige TK, Linder SW. Capillary electrophoresis/inductively-coupled plasma-mass spectrometry: development and optimization of a high resolution analytical tool for the size-based characterization of nanomaterials in dietary supplements. Anal Chem. 2014;86:11620–7.

Quadros ME, Marr LC. Silver nanoparticles and Total aerosols emitted by nanotechnology-related consumer spray products. Environ Sci Technol. 2011;45:10713–9.

Quik JTK, Vonk AI, Hansen SF, Baun A, Van De Meent D. How to assess exposure of aquatic organisms to manufactured nanoparticles? Environ Int. 2011;37(6):1068–77.

Salieri B, Turner B, Nowack B, Hischler R. Life cycle assessment of manufactured nanomaterials: where are we? NanoImpact. 2018;10:108–20.

TACD (Trans Atlantic Consumer Dialogue). Resolution on the need for mandatory reporting scheme and inventory for nanomaterials contained in consumer products. DOC No. Nano 02-11. 2011.

The Nanodatabase. The nanodatabase. 2017. Available: www.nanodb.dk. Accessed 15 Jan 2017.

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF Jr, Rejeski D, Hull MS. Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory. Beilstein J Nanotechnol. 2015;6:1769–80.

von Goetz N, Fabricius L, Glaus R, Weitbrecht V, Gunther D, Hungerbuhler K. Migration of silver from commercial plastic food containers and implications for consumer exposure assessment. Food Addit Contam. 2013;30:612–20.

Wagener S, Dommershausen N, Jungnickel H, Laux P, Mitrano D, Nowack B, Schneider G, Luch A. Textile functionalization and its effects on the release of silver nanoparticles into artificial sweat. Environ Sci Technol. 2016;50:5927–34.

Walser T, Limbach LK, Brogioli R, et al. Persistence of engineered nanoparticles in a municipal solid-waste incineration plant. Nat Nanotechnol. 2012;7:520–4.

Wijnhoven SWP, Oomen AG, Sips AJAM, Bourgeois FC, te Dorsthorst GJPM, Kooi MW, Bakker MI. Development of an inventory for consumer products containing nanomaterials. Final Report 070307/2010/580587/SER/D3. 2010. Available: http://ec.europa.eu/environment/chemicals/nanotech/pdf/study_inventory.pdf. Accessed 29 Oct 2015.

Windler L, Lorenz C, Von Goetz N, Hungerbühler K, Amberg M, Heuberger M, Nowack B. Release of titanium dioxide from textiles during washing. Environ Sci Technol [Internet]. 2012 [cited 2016 May 20];46:8181–8. Available from: http://pubs.acs.org/doi/abs/10.1021/es301633b

Zainzinger V. "Anomalies" in notifications behind nanomaterials inventory delay Cosmetics industry confused by different national rules, lack of test methods. 5 Mar 2015. Available: https://chemicalwatch.com/23044/anomalies-in-notifications-behind-nanomaterials-inventory-delay?q=nano%2C%20inventory. Accessed 13 Aug 2015.

Factors Affecting Nanoparticle Dose–Exposure and Cell Response

Sandor Balog, Barbara Rothen-Rutishauser, and Alke Petri-Fink

Contents

Abstract To understand cell response and the dose-dependent interaction between particles and cells, in vitro dosimetry of nanoparticles has become an important concept in predicting the dose delivered to cells that are cultured in plastic wells. The usefulness of any dosimetry method, however, requires a throughout experimental characterization of the physicochemical properties of the particles in the dispersion under study. Here we present the major aspects one must carefully consider and adapt in order to obtain reliable and reproducible results.

Keywords In vitro dosimetry · Nanoparticles · Dispersion · Physicochemical properties

Introduction

Nanotechnology enables engineering nanoparticles (NPs) with desired physicochemical properties useful for a wide range of applications. NPs are materials with any external dimension on the nanoscale and properties differing from their bulk

S. Balog · B. Rothen-Rutishauser
Adolphe Merkle Institute, Fribourg, Switzerland

A. Petri-Fink (✉)
Adolphe Merkle Institute, Fribourg, Switzerland

Department of Chemistry, University of Fribourg, Fribourg, Switzerland
e-mail: alke.fink@unifr.ch

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_5

equivalents, which allows for novel applications in almost any (industrial) sector. This huge potential has led to an increasing growth of research and development activities and created an entire new class of materials which are used in a broad field of applications such as in optics, electronics (e.g. for efficient and cost-effective energy storage or their use as semiconductors) (Jariwala et al. 2013), and in the medical field as potential carriers for drug and gene delivery or as diagnostic tools and contrast agents (De Jong and Borm 2008).

However, these new properties and the increasing industrial production have raised concerns about potential adverse effects for human health; thus, a better understanding of cellular consequences upon the direct exposure of (human) cells to NPs is prerequisite for their safe-by-design and successful use in any applications. New concepts for efficient, cheaper and evidence-based testing strategies were proposed, based on the in vitro use of human primary cells and cell lines (Krewski et al. 2010; Drasler et al. 2017a).

In order that the outcomes of in vitro studies can be useful in a regulatory context, the results must be transformed to concentrations that are meaningful in real scenarios. Concentrations of NPs and their respective doses should be realistic, i.e. relevant to human exposure scenarios such as occupational exposure limits for NPs (Gordon et al. 2014), e.g. using a model for calculation of the deposited dose based on the concentration in the dispersion or suspension administered (Hinderliter et al. 2010).

When interpreting data from studies within nanotoxicology, attention should be paid to the administered concentration and cellular delivered doses since discrepancy between the administered and deposited doses might be substantial. The deposited dose can be evaluated with dosimetry models or by experimentally assessing NP concentration on the cells or intracellularly, for instance with mass spectroscopy-based techniques (Drasler et al. 2017b).

Physicochemical Properties of Nanoparticle Dispersions and Suspensions

Classical particle systems are essentially biphasic colloidal systems where the matrix phase is a liquid, e.g. a buffered aqueous solution in which the solid-phase, organic, inorganic or hybrid nanoparticles (NPs) are dispersed or suspended. By dispersion one usually refers to a colloidal system where particles do not settle due to gravity (even over a long period of time), and by suspension, to systems where the particles settle due to gravity, which would be a typical case of aggregates and agglomerates of NPs.

The primary chemical and physical properties of NPs are particle concentration, particle size and size distribution, particle shape and shape uniformity, chemical composition of the core and surface and their relative proportions of components, surface density and conformation of functional groups on the surface, phases found in/on the particle (such as amorphous and crystalline phases and purity of phases),

porosity and structure of porosity. From these primary properties derived are the secondary properties, such as elasticity, volume-averaged mass density, hydrodynamic size, and effective surface charge. All these factors determine physical stability and chemical integrity (e.g. dissolution, ligand exchange) and also colloidal stability in terms of aggregation and agglomeration. While the underlying physical principles of the formation of aggregates and agglomerates are identical, from the point of view nanoparticle dose–exposure and cell response, one must still distinguish these two terms describing particle clusters. While each term has its specific meaning, they are frequently interchanged (Nichols et al. 2002): Agglomerates correspond to particles held together by a relatively weak net inter-particle force, and accordingly the cluster formation is reversible. For example, agglomerates can be broken up easily by shaking or sonicating the sample, and temporary colloidal stability can be restored by e.g. simply diluting the sample. In contrast, aggregates are held together by strong net inter-particle forces that the clustering process is irreversible via the means mentioned before, and colloidal stability cannot be restored. Briefly, aggregation is an irreversible process, whilst agglomeration is a reversible process (Sokolov et al. 2015).

Therefore, colloidal stability refers to particle systems that do not aggregate. Stability is essential if one is interested in studying the physiochemical properties and their surface activity relating to the exceptionally high surface-to-volume ratio NPs can provide. To achieve permanent stability, one must ensure that repulsive inter-particle forces dominate attractive forces. Systems that agglomerate can offer only temporary stability, because of the weak but attractive net inter-particle force that drives the formation of clusters. Given that the strengths of these forces (a) are functions of the distance between particles, and (b) inter-particle distance is a function of the concentration, colloidal stability can be considered concentration dependent. A typical case is a dispersion that is stable at low concentration (e.g. below a few μg/mL), but aggerates quickly at higher concentrations. The most important thing is to always keep in mind that primary particles and their aggregates (even if aggregation is reversible) are definitely not identical systems. A typical example could be the case of superparamagnetic NPs: when they are well-dispersed they exhibit magnetic properties that their aggregates cannot reproduce (Jeon et al. 2016). Accordingly, describing the particles in regard to colloidal stability, extent of aggregation, is of primary importance (Faria et al. 2018), albeit not without difficulties (Miernicki et al. 2019). Furthermore, any feature that might be useful for potential applications—such as optical, thermal, magnetic, mechanical and catalytic characteristics—will be eventually defined by the primary and secondary properties mentioned above, and consequently, the behaviour and physicochemical properties of a NP cannot be decoupled from the matrix.

From this aspect, physiological and biological fluids—such as cell culture media, intra-and extracellular fluid, plasma and blood—themselves are truly complex colloidal media (Urban et al. 2016), for they are mixtures of substances either dissolved or dispersed or suspended in water. They contain of a multitude of components and are generally rich in ions and proteins, which may affect and completely transform the surface-related physicochemical properties of the NPs (Fong et al. 2019).

For example, gold NPs aggregate in water due to the van der Waals forces and the high Hamaker constant, but they become colloidally stable in human plasma, owing to the proteins that adsorb onto the surface, resulting in repulsive and thus stabilizing inter-particle forces (Michen et al. 2015). With other particles the opposite is frequent, and aggregation is rapid upon dispersion in complex media (Balog et al. 2015), because for example the electric double layer originating from the particle's surface charge can collapse in the presence of an electrolyte. Dissolution of silver NPs—owing to the presence of an oxidizing agent—is a typical case of loss of physical integrity that transforms chemical reactivity as well (Loza et al. 2014). To summarize, there are material properties that do not change in the matrix interacting with the NPs, e.g. crystallinity and shape of the particle core. If such 'intrinsic' properties change, it is considered as a 'transformation' of the NPs, and it is often irreversible. There are properties that change easily, often reversibly. A typical example is surface charge and related zeta-potential.

What exactly happens to a particle system and to what extent in a complex medium is generally not trivial to predict, for it depends on the initial volumetric and surface properties of the NPs and on the components of the complex medium. Accordingly, the characterisation by experimental means should be able to resolve particle properties in either scenario. The major techniques either directly dedicated or adapted to the physicochemical characterisation of NPs in complex media are described elsewhere (Lin et al. 2014; Treuel et al. 2014).

It is important to stress that changes that happen to NPs are not always instantaneous, but can take time, e.g. the reaction kinetics of dissolution can be very slow, and the rate of change is in general dependent on the concentration of the components taking part in the reaction. Therefore, characterization of 'particle transformation' should be also able to capture the course of time-dependency. Only when the physical and chemical properties and their course in time are characterized, one can begin the dose-exposure-response analyses.

Administered, Delivered and Cellular Dose

Here we focus on factors that are relevant to in vitro submerged cell cultures assays (Drasler et al. 2017a), applying concepts developed in cell biology, hazard assessment and pharmacology. Exposure refers to any situation that provides an opportunity for the given particle type to interact with the cell capable of adhering to the outer cell membrane, being internalised and possibly eliciting a biological response. Dose quantifies the number of NPs that actually come in contact with the cell. Accordingly, the dose-response assessment requires a realistic and quantitative characterization of the relationships between varying doses and magnitude of adverse effects in exposed populations.

In this relationship, at least three levels of doses can be distinguished: administered dose which is usually given as a particle concentration, delivered dose to the cell surface, and intracellular dose. In this order increases the difficulty to reliably

determine these three distinct measures, and the metric may be multi-fold. The most basic ones are number, mass, and surface area of the NPs, but given the compositional versatility, this primary list can be quickly expanded by e.g. charge, ligand type, conformation, charge distribution and density (Burnand et al. 2018). While the conversion from one to the other is relatively straightforward when NPs are uniform, polydispersity in size and heterogeneity in shape rapidly eliminates this convenience for average values may not be sufficiently intricate. Therefore, the experimental characterization should be able to account for these variables as well as their conversion. Additionally, another aspect is to consider is the fact that many experimental techniques do not probe the primary quantities, but rely on physicochemical phenomena and measurable quantities to estimate primary quantities via e.g. mathematical relationships (Gao and Lowry 2018). For example, optical extinction of gold and silver NPs may be used to estimate particle size and concentration via the phenomenon of localised surface plasmon resonance, yet the accuracy may be limited by particle polydispersity and shape heterogeneity—even if the particles are nominally spherical (Haiss et al. 2007).

The administered dose is the totality of the quantity of interest and is directly proportional to the concentration of the particles added to the adherent cell cultures. The delivered dose refers to the quantity that reaches the cell and is able to interact with. The delivered dose is defined by the rate NP arrive to the cell level (e.g. μg/h), via translational diffusion and gravitational settling, which may be followed by adhering to the outer cell membrane and subsequent internalization. The last step defines the cellular dose.

The delivered dose is dependent on the time of exposure and on the hydrodynamic properties of the NPs that defines the rate of translational diffusion and sedimentation velocity, which both are functions the particle hydrodynamic radius and effective mass density. When using the correct hydrodynamic model for the observed particle shape obtained by e.g. transmission electron microscopy (TEM) characterization, one can successfully predict the hydrodynamic properties of the particles (Martchenko et al. 2011) and they can be also obtained directly by experimental means, for example via analytical ultracentrifugation (Silvera Batista et al. 2014; Walter et al. 2014, 2017; Bekdemir and Stellacci 2016; Thajudeen et al. 2017), centrifugal sedimentation (Davidson et al. 2017; Minelli et al. 2018), and dynamic light scattering (Boluk and Danumah 2013; Balog et al. 2014; Stoehr et al. 2015; Geers et al. 2016; Bossert et al. 2017, 2018).

The cellular dose is a more complex function of several other variables, because the physicochemical properties of the particle can play a fundamental role in endocytosis, which may involve a variety of mechanisms. Until now shape, volume, elasticity, overall surface chemistry, spatial arrangement and surface density of ligands, adhesion strength to cell membrane, cell type, cell cycle, and the environment and experimental conditions have been recognized to the influence intracellular dose (Burnand et al. 2018; Zhang et al. 2008; Jiang et al. 2008; Verma et al. 2008; Nel et al. 2009; Yuan et al. 2010; Yuan and Zhang 2010; Summers et al. 2011; Kato 2011; Kim et al. 2011; Albanese et al. 2012; Walkey et al. 2012; Kettler et al. 2013; Huang et al. 2013; Zhang et al. 2015; Li et al. 2015, 2017; Anselmo and

Mitragotri 2017; Yi and Gao 2017). Additionally, particles may aggregate, may be dissolved, may either lose or replace their ligands, which further adds to the complexity of this subject (Dale et al. 2017; Hirsch et al. 2014).

It is not less important that even the way one administers particles influences experimental outcomes describing particle-cell interactions (Moore et al. 2019). It has been demonstrated by Moore et al. that the initial formation of the protein corona may be different, depending on whether the total administered dose is a concentrated bolus of particles or particles pre-mixed in complete cell culture media prior to cell exposure (Moore et al. 2019). Given that particle size and surface properties determine the protein corona as well: its composition and 'layer-cake-like' structure (Lundqvist et al. 2008), the high degree of variations and subsequent combinations between (a) particle properties, (b) techniques of particle administration, (c) composition and concentration of proteins, as well as (d) cell type may result in a large variety of different protein coronas, and accordingly, a large variety in the rates of cellular adsorption and uptake (Walkey et al. 2012; Moore et al. 2019), even if the experiments were most diligently executed with minimal error and bias.

The delivery of particle dose can be reliably modelled by knowing the hydrodynamic properties of the particle system (whether it is single particles or particle aggregates), which is highly relevant when considering that the interactions between particles and cells are extremely intricate. However, it is important to point out that the rate of particle internalization may also define the delivered dose itself: while large particles, whose transport to the cell-level is driven dominantly by gravitational settling, will be delivered to the cell no matter whether uptake happens or not, diffusion-driven transport of small NPs is induced by concentration gradient created by cellular uptake, and when cells do not take up particles, delivery does not occur. Such aspects render it difficult to model and predict the rate of internalization, and consequently, the knowledge of the delivered dose is essential when deciphering interactions between cells and particles (Faria et al. 2019).

Indeed, the fundamental role of delivered particle dose for translating toxicological dose-response data into risk assessment techniques and exposure limits cannot be stressed enough, for it is of overarching significance for any type of particle exposure scenario (Schmid and Cassee 2017). Therefore, state-of-the-art computational models aim to provide standardized protocols that integrate the necessary steps and algorithms to estimate in vitro delivered dose (Hinderliter et al. 2010; Teeguarden et al. 2007; Mahnama et al. 2014; Rodriguez-Lorenzo et al. 2015; DeLoid et al. 2015, 2017; Thomas et al. 2018; Johnston et al. 2018; Price et al. 2019; Johnston et al. 2020, 2021; Balog et al. 2021; Böhmert et al. 2018; Frenzel et al. 2020). The concept stems from the recognition that in in vitro cell culture experiments, dilute suspensions and dispersion are used in the absence of fluid flow, and therefore, the Reynolds number is small. In such dilute and quiescent colloidal dispersions/suspensions there is no collective fluid motion, and viscous forces dominate over inertial forces, and interparticle interactions are negligible. Accordingly, the transport-relevant particle property is a function of two parameters: hydrodynamic radius and mass density, and is quantified by the diffusion coefficient (the Stokes–Einstein equation) and the settling velocity (the Stokes' law). Estimating the

delivered dose profile helps in planning the overall time span of the exposure and particle delivery, defining endpoints, and defining the administered concentration of particles according to the area of the cell-culture and the depth of the medium covering the cells.

Therefore, the knowledge of the delivered dose becomes essential in the interpretation of any experimental data addressing the interaction between cells and particles. Nonetheless, there are particle systems that are truly heterogeneous and ill-defined in size, shape and composition (such as high aspect ratio materials, i.e. carbon nanotubes, gold nanorods), and characterization provides very limited information on averages. In this case, a direct estimation of delivered dose should be achieved by experimental means, via, for example, UV-Vis spectroscopy (Rischitor et al. 2016).

To summarize, in this chapter we outlined the major concepts one must carefully address (also illustrated in Fig. 1) when interpreting nanoparticle dose–exposure and cell response. These concepts are essential guidelines leading to a multidisciplinary effort converging on analytics, and one must always keep in mind that the devil is in the detail. Indeed, like any guideline, ours may shed light on the cruciality of good analytics, but it cannot confer experience and awareness. Given that each particle material and physiological / biological environment may present their own box full of challenges, analytical protocols must be most carefully designed accordingly.

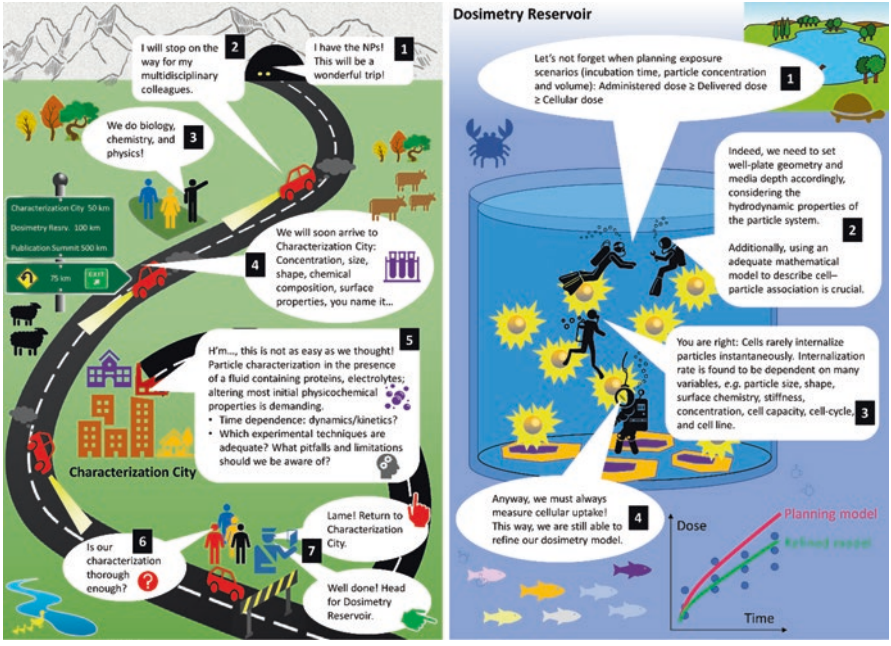


Fig. 1 A schematic of the scenic road any scientist working on the nanomaterial-cell interaction must take for in vitro dosimetry and related particle system characterization

Outlook

In order to develop nanoparticle systems that are safe by design, another aspect is opening the field (open science), and make research data, software and other tools to analyse data freely available. This could permit verifying and perhaps even improving obtained results in comparison to the approaches followed by the original research team, and also promote transparency and reproducibility (Leong et al. 2019; Nat Nanotechnol 2019).

Not to mention but one example, intelligent machine systems are able to explore the volume of (big) data beyond human capacity and can discover off-target or hidden relationships that were not addressed in the original analyses (Labouta et al. 2019).

Acknowledgement The authors are grateful for the financial support of the Adolphe Merkle Foundation, the University of Fribourg, and the Swiss National Science Foundation through the National Centre of Competence in Research Bio-Inspired Materials. The authors are also grateful to Dr. Barbara Drasler for carefully reading the chapter.

References

Albanese A, Tang PS, Chan WCW. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu Rev Biomed Eng. 2012;14:1–16.

Anselmo AC, Mitragotri S. Impact of particle elasticity on particle-based drug delivery systems. Adv Drug Deliv Rev. 2017;108:51–67.

Balog S, Rodriguez-Lorenzo L, Monnier CA, Michen B, Obiols-Rabasa M, Casal-Dujat L, Rothen-Rutishauser B, Petri-Fink A, Schurtenberger P. Dynamic depolarized light scattering of small round plasmonic nanoparticles: when imperfection is only perfect. J Phys Chem C. 2014;118:17968–74.

Balog S, Rodriguez-Lorenzo L, Monnier CA, Obiols-Rabasa M, Rothen-Rutishauser B, Schurtenberger P, Petri-Fink A. Characterizing nanoparticles in complex biological media and physiological fluids with depolarized dynamic light scattering. Nanoscale. 2015;7:5991–7.

Balog S; Rothen-Rutishauser B, Fink A. Fluid menisci and in vitro particle dosimetry of submerged cells. bioRxiv. 2021, 2021.2003.2025.436962.

Bekdemir A, Stellacci F. A centrifugation-based physicochemical characterization method for the interaction between proteins and nanoparticles. Nat Commun. 2016;7:13121.

Böhmert L, König L, Sieg H, Lichtenstein D, Paul N, Braeuning A, Voigt A, Lampen A. In vitro nanoparticle dosimetry for adherent growing cell monolayers covering bottom and lateral walls. Part Fibre Toxicol. 2018;15:42.

Boluk Y, Danumah C. Analysis of cellulose nanocrystal rod lengths by dynamic light scattering and electron microscopy. J Nanopart Res. 2013;16:2174.

Bossert D, Natterodt J, Urban DA, Weder C, Petri-Fink A, Balog S. Speckle-visibility spectroscopy of depolarized dynamic light scattering. J Phys Chem B. 2017;121:7999–8007.

Bossert D, Crippa F, Petri-Fink A, Balog S. Hypothesis test of the photon count distribution for dust discrimination in dynamic light scattering. Anal Chem. 2018;90:3656–60.

Burnand D, Milosevic A, Balog S, Spuch-Calvar M, Rothen-Rutishauser B, Dengjel J, Kinnear C, Moore TL, Petri-Fink A. Beyond global charge: role of amine bulkiness and protein fingerprint on nanoparticle–cell interaction. Small. 2018;14:1802088.

Dale AL, Lowry GV, Casman EA. Accurate and fast numerical algorithms for tracking particle size distributions during nanoparticle aggregation and dissolution. Environ Sci Nano. 2017;4:89–104.

Davidson AM, Brust M, Cooper DL, Volk M. Sensitive analysis of protein adsorption to colloidal gold by differential centrifugal sedimentation. Anal Chem. 2017;89:6807–14.

De Jong WH, Borm PJA. Drug delivery and nanoparticles: applications and hazards. Int J Nanomedicine. 2008;3:133–49.

DeLoid GM, Cohen JM, Pyrgiotakis G, Pirela SV, Pal A, Liu J, Srebric J, Demokritou P. Advanced computational modeling for in vitro nanomaterial dosimetry. Part Fibre Toxicol. 2015;12:32.

DeLoid GM, Cohen JM, Pyrgiotakis G, Demokritou P. Preparation, characterization, and in vitro dosimetry of dispersed, engineered nanomaterials. Nat Protoc. 2017;12:355.

Drasler B, Sayre P, Steinhäuser KG, Petri-Fink A, Rothen-Rutishauser B. In vitro approaches to assess the hazard of nanomaterials. NanoImpact. 2017a;8:99–116.

Drasler B, Vanhecke D, Rodriguez-Lorenzo L, Petri-Fink A, Rothen-Rutishauser B. Quantifying nanoparticle cellular uptake: which method is best? Nanomedicine. 2017b;12:1095–9.

Faria M, Björnmalm M, Thurecht KJ, Kent SJ, Parton RG, Kavallaris M, Johnston APR, Gooding JJ, Corrie SR, Boyd BJ, Thordarson P, Whittaker AK, Stevens MM, Prestidge CA, Porter CJH, Parak WJ, Davis TP, Crampin EJ, Caruso F. Minimum information reporting in bio–nano experimental literature. Nat Nanotechnol. 2018;13:777–85.

Faria M, Noi KF, Dai Q, Björnmalm M, Johnston ST, Kempe K, Caruso F, Crampin EJ. Revisiting cell–particle association in vitro: a quantitative method to compare particle performance. J Control Release. 2019;307:355–67.

Fong W-K, Moore TL, Balog S, Vanhecke D, Rodriguez-Lorenzo L, Rothen-Rutishauser B, Lattuada M, Petri-Fink A. In: Gehr P, Zellner R, editors. Nanoparticle Behaviour in Complex Media: Methods for Characterizing Physicochemical Properties, Evaluating Protein Corona Formation, and Implications for Biological Studies. Cham: Springer International Publishing; 2019. p. 101–50.

Frenzel F, König-Mattern L, Stock V, Voss L, Paul MB, Sieg H, Braeuning A, Voigt A, Böhmert L. Nanopass: an easy-to-use user interface for nanoparticle dosimetry with the 3dsdd model. Part Fibre Toxicol. 2020;17:45.

Gao X, Lowry GV. Progress towards standardized and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks. NanoImpact. 2018;9:14–30.

Geers C, Rodriguez-Lorenzo L, Andreas Urban D, Kinnear C, Petri-Fink A, Balog S. A new angle on dynamic depolarized light scattering: number-averaged size distribution of nanoparticles in focus. Nanoscale. 2016;8:15813–21.

Gordon SC, Butala JH, Carter JM, Elder A, Gordon T, Gray G, Sayre PG, Schulte PA, Tsai CS, West J. Workshop report: strategies for setting occupational exposure limits for engineered nanomaterials. Regul Toxicol Pharmacol. 2014;68:305–11.

Haiss W, Thanh NTK, Aveyard J, Fernig DG. Determination of size and concentration of gold nanoparticles from Uv−Vis spectra. Anal Chem. 2007;79:4215–21.

Hinderliter PM, Minard KR, Orr G, Chrisler WB, Thrall BD, Pounds JG, Teeguarden JG. Isdd: a computational model of particle sedimentation, diffusion and target cell dosimetry for in vitro toxicity studies. Part Fibre Toxicol. 2010;7:36.

Hirsch V, Kinnear C, Rodriguez-Lorenzo L, Monnier CA, Rothen-Rutishauser B, Balog S, Petri-Fink A. In vitro dosimetry of agglomerates. Nanoscale. 2014;6:7325–31.

Huang C, Butler PJ, Tong S, Muddana HS, Bao G, Zhang S. Substrate stiffness regulates cellular uptake of nanoparticles. Nano Lett. 2013;13:1611–5.

Jariwala D, Sangwan VK, Lauhon LJ, Marks TJ, Hersam MC. Carbon nanomaterials for electronics, optoelectronics, photovoltaics, and sensing. Chem Soc Rev. 2013;42:2824–60.

Jeon S, Hurley KR, Bischof JC, Haynes CL, Hogan CJ. Quantifying intra- and extracellular aggregation of iron oxide nanoparticles and its influence on specific absorption rate. Nanoscale. 2016;8:16053–64.

Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. Nat Nanotechnol. 2008;3:145.

Johnston ST, Faria M, Crampin EJ. An analytical approach for quantifying the influence of nanoparticle polydispersity on cellular delivered dose. J R Soc Interface. 2018;15:20180364.

Johnston ST, Faria M, Crampin EJ. Isolating the sources of heterogeneity in nano-engineered particle-cell interactions. J R Soc Interface. 2020;17:20200221.

Johnston ST, Faria M, Crampin EJ. Understanding nano-engineered particle–cell interactions: biological insights from mathematical models. Nanoscale Adv. 2021;3:2139–56.

Kato H. Tracking nanoparticles inside cells. Nat Nanotechnol. 2011;6:139.

Kettler K, Veltman K, van de Meent D, van Wezel A, Hendriks AJ. Cellular uptake of nanoparticles as determined by particle properties, experimental conditions, and cell type. Environ Toxicol Chem. 2013;33:481–92.

Kim JA, Åberg C, Salvati A, Dawson KA. Role of cell cycle on the cellular uptake and dilution of nanoparticles in a cell population. Nat Nanotechnol. 2011;7:62.

Krewski D, Acosta D Jr, Andersen M, Anderson H, Bailar JC 3rd, Boekelheide K, Brent R, Charnley G, Cheung VG, Green S Jr, Kelsey KT, Kerkvliet NI, Li AA, McCray L, Meyer O, Patterson RD, Pennie W, Scala RA, Solomon GM, Stephens M, et al. Toxicity testing in the 21st century: a vision and a strategy. J Toxicol Environ Health B Crit Rev. 2010;13:51–138.

Labouta HI, Asgarian N, Rinker K, Cramb DT. Meta-analysis of nanoparticle cytotoxicity via data-mining the literature. ACS Nano. 2019;13:1583–94.

Leong HS, Butler KS, Brinker CJ, Azzawi M, Conlan S, Dufés C, Owen A, Rannard S, Scott C, Chen C, Dobrovolskaia MA, Kozlov SV, Prina-Mello A, Schmid R, Wick P, Caputo F, Boisseau P, Crist RM, McNeil SE, Fadeel B, et al. On the issue of transparency and reproducibility in nanomedicine. Nat Nanotechnol. 2019;14:629–35.

Li Y, Zhang X, Cao D. Nanoparticle hardness controls the internalization pathway for drug delivery. Nanoscale. 2015;7:2758–69.

Li L, Zhang Y, Wang J. Effects of ligand distribution on receptor-diffusion-mediated cellular uptake of nanoparticles. R Soc Open Sci. 2017;4:170063.

Lin P-C, Lin S, Wang PC, Sridhar R. Techniques for physicochemical characterization of nanomaterials. Biotechnol Adv. 2014;32:711–26.

Loza K, Diendorf J, Sengstock C, Ruiz-Gonzalez L, Gonzalez-Calbet JM, Vallet-Regi M, Köller M, Epple M. The dissolution and biological effects of silver nanoparticles in biological media. J Mater Chem B. 2014;2:1634–43.

Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein Corona with possible implications for biological impacts. Proc Natl Acad Sci. 2008;105:14265.

Mahnama A, Ghorbaniasl G, Allaei SMV, Nourbakhsh A. Semi-analytical solution for the in-vitro sedimentation, diffusion and dosimetry model: surveying the impact of the Peclet number. Colloids Surf B: Biointerfaces. 2014;122:324–31.

Martchenko I, Dietsch H, Moitzi C, Schurtenberger P. Hydrodynamic properties of magnetic nanoparticles with tunable shape anisotropy: prediction and experimental verification. J Phys Chem B. 2011;115:14838–45.

Michen B, Geers C, Vanhecke D, Endes C, Rothen-Rutishauser B, Balog S, Petri-Fink A. Avoiding drying-artifacts in transmission electron microscopy: characterizing the size and colloidal state of nanoparticles. Sci Rep. 2015;5:9793.

Miernicki M, Hofmann T, Eisenberger I, von der Kammer F, Praetorius A. Legal and practical challenges in classifying nanomaterials according to regulatory definitions. Nat Nanotechnol. 2019;14:208–16.

Minelli C, Sikora A, Garcia-Diez R, Sparnacci K, Gollwitzer C, Krumrey M, Shard AG. Measuring the size and density of nanoparticles by centrifugal sedimentation and flotation. Anal Methods. 2018;10:1725–32.

Moore TL, Urban DA, Rodriguez-Lorenzo L, Milosevic A, Crippa F, Spuch-Calvar M, Balog S, Rothen-Rutishauser B, Lattuada M, Petri-Fink A. Nanoparticle administration method in cell culture alters particle-cell interaction. Sci Rep. 2019;9:900.

Nel AE, Mädler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater. 2009;8:543.

Nichols G, Byard S, Bloxham MJ, Botterill J, Dawson NJ, Dennis A, Diart V, North NC, Sherwood JD. A review of the terms agglomerate and aggregate with a recommendation for nomenclature used in powder and particle characterization. J Pharm Sci. 2002;91:2103–9.

Price SR, Kinnear C, Balog S. Particokinetics and in vitro dose of high aspect ratio nanoparticles. Nanoscale. 2019;11:5209–14.

Rischitor G, Parracino M, La Spina R, Urbán P, Ojea-Jiménez I, Bellido E, Valsesia A, Gioria S, Capomaccio R, Kinsner-Ovaskainen A, Gilliland D, Rossi F, Colpo P. Quantification of the cellular dose and characterization of nanoparticle transport during in vitro testing. Part Fibre Toxicol. 2016;13:47.

Rodriguez-Lorenzo L, Rothen-Rutishauser B, Petri-Fink A, Balog S. Nanoparticle polydispersity can strongly affect in vitro dose. Part Part Syst Charact. 2015;32:321–33.

Schmid O, Cassee FR. On the pivotal role of dose for particle toxicology and risk assessment: exposure is a poor surrogate for delivered dose. Part Fibre Toxicol. 2017;14:52.

Silvera Batista CA, Zheng M, Khripin CY, Tu X, Fagan JA. Rod hydrodynamics and length distributions of single-wall carbon nanotubes using analytical ultracentrifugation. Langmuir. 2014;30:4895–904.

Sokolov SV, Tschulik K, Batchelor-McAuley C, Jurkschat K, Compton RG. Reversible or not? Distinguishing agglomeration and aggregation at the nanoscale. Anal Chem. 2015;87:10033–9.

Stoehr LC, Endes C, Radauer-Preiml I, Boyles MS, Casals E, Balog S, Pesch M, Petri-Fink A, Rothen-Rutishauser B, Himly M, Clift MJ, Duschl A. Assessment of a panel of interleukin-8 reporter lung epithelial cell lines to monitor the pro-inflammatory response following zinc oxide nanoparticle exposure under different cell culture conditions. Part Fibre Toxicol. 2015;12:29.

Summers HD, Rees P, Holton MD, Rowan Brown M, Chappell SC, Smith PJ, Errington RJ. Statistical analysis of nanoparticle dosing in a dynamic cellular system. Nat Nanotechnol. 2011;6:170.

Teeguarden JG, Hinderliter PM, Orr G, Thrall BD, Pounds JG. Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments. Toxicol Sci. 2007;95:300–12.

Thajudeen T, Walter J, Srikantharajah R, Lübbert C, Peukert W. Determination of the length and diameter of nanorods by a combination of analytical ultracentrifugation and scanning mobility particle sizer. Nanoscale Horizons. 2017;2:253–60.

Thomas DG, Smith JN, Thrall BD, Baer DR, Jolley H, Munusamy P, Kodali V, Demokritou P, Cohen J, Teeguarden JG. Isd3: a Particokinetic model for predicting the combined effects of particle sedimentation, diffusion and dissolution on cellular dosimetry for in vitro systems. Part Fibre Toxicol. 2018;15:6.

Treuel L, Eslahian KA, Docter D, Lang T, Zellner R, Nienhaus K, Nienhaus GU, Stauber RH, Maskos M. Physicochemical characterization of nanoparticles and their behavior in the biological environment. Phys Chem Chem Phys. 2014;16:15053–67.

Urban DA, Rodriguez-Lorenzo L, Balog S, Kinnear C, Rothen-Rutishauser B, Petri-Fink A. Plasmonic nanoparticles and their characterization in physiological fluids. Colloids Surf B: Biointerfaces. 2016;137:39–49.

Verma A, Uzun O, Hu Y, Hu Y, Han H-S, Watson N, Chen S, Irvine DJ, Stellacci F. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. Nat Mater. 2008;7:588.

Voices from the community. Nat Nanotechnol. 2019;14:625–5.

Walkey CD, Olsen JB, Guo H, Emili A, Chan WCW. Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake. J Am Chem Soc. 2012;134:2139–47.

Walter J, Löhr K, Karabudak E, Reis W, Mikhael J, Peukert W, Wohlleben W, Cölfen H. Multidimensional analysis of nanoparticles with highly disperse properties using multi-wavelength analytical ultracentrifugation. ACS Nano. 2014;8:8871–86.
Walter J, Gorbet G, Akdas T, Segets D, Demeler B, Peukert W. 2d analysis of polydisperse core–shell nanoparticles using analytical ultracentrifugation. Analyst. 2017;142:206–17.
Yi X, Gao H. Kinetics of receptor-mediated endocytosis of elastic nanoparticles. Nanoscale. 2017;9:454–63.
Yuan H, Zhang S. Effects of particle size and ligand density on the kinetics of receptor-mediated endocytosis of nanoparticles. Appl Phys Lett. 2010;96:033704.
Yuan H, Li J, Bao G, Zhang S. Variable nanoparticle-cell adhesion strength regulates cellular uptake. Phys Rev Lett. 2010;105:138101.
Zhang S, Li J, Lykotrafitis G, Bao G, Suresh S. Size-dependent endocytosis of nanoparticles. Adv Mater. 2008;21:419–24.
Zhang S, Gao H, Bao G. Physical principles of nanoparticle cellular endocytosis. ACS Nano. 2015;9:8655–71.

Mapping Exposure onto Nanoscale Toxicity Measures

Daniel A. Vallero

Contents

Abstract Risk assessment consists of investigations of both the hazard of an agent and the likelihood that the agent will come into contact with a human or other target organism. This chapter introduces exposure assessment as it applies to predicted exposure concentrations for substances generated by emerging technologies, especially nanotechnology. It also identifies areas where assessing exposure to emerging technologies may differ from conventional exposure assessments.

Keywords Exposure · Dosimetry · Aerosol · Gas-phase · Nanoparticle · Nanomaterial · Refererence concentration (RfC) · Dose-response curve · Risk assessment · Particulate matter (PM) · Gas-phase pollutants · Adverse outcome pathway (AOP) · Toxicokinetics

D. A. Vallero (✉)
U.S. Environmental Protection Agency, Center for Computational Toxicology and Exposure, Research Triangle Park, NC, USA
e-mail: vallero.daniel@epa.gov

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_6

Introduction

Risk is a common metric for public health and environmental decision making. Scientifically credible risk assessments must underpin decisions regarding the potential safety of emerging technologies (National Research Council 1983). Furthermore, those who may be exposed to the nanoparticles and other constituents of the products from these technologies must understand and predict the exposure to allow them decide whether the pending risks are acceptable. Most enviromental exposure decisions have a low probability of risk, e.g. purchase of pollution control and personal protective equipment. Many, perhaps most, personal decisions made each day relate to risk, for example:

- Can the risk of cancer be reduced by what one has for breakfast?
- Do the ingredients in ths shampoo add to one's risk of allergic reactions?
- Is riding a bike to work safe enough to arrive safely at a destination while reducing one's carbon footprint (risk of exacerabing climate change)?
- Is it too risky to use this pesticide?

When such decisions that involve emerging technologies like nanotechnology and synthetic biology the consumer has even greater uncertainty than these everyday and common exposure scenarios. The policy maker and regulator deal with such uncertainty with incomplete data, often relying on comparisions to conventional technologies. However, the flaw in this logic is that there may be substantive differences in the physical, chemical and biological properties of contaminants produced from nano-scale processes.

The more advanced the technology, the more uncertain the potential hazard, exposure and risk will be. Uncertainty has at least three dimensions: scientific; regulatory; and attitudinal (Arnaldi and Muratorio 2013). From a scientific perspective, most of the data and information available to assess risk are from experiments in tightly controlled settings (Poel 2009). Real-life expsoure scenarios have myriad factors that vary in space and time from the experiments, increasing the likelihood that most important variables are untested and unforeseen. Regulatory uncertainty stems from the diversity in how various governances attempt to control and prevent the risks. Some agencies emphasize precautionary approaches, others awaiting reliable evidence of harm (Falkner and Jaspers 2012). The third dimension, risk perception, is highly variable even for conventional pollutants, but uncertainty of the risks posed by nanotechnology increases the social aversion (Linkov et al. 2006; Satterfield et al. 2009).

Traditional exposure events have track records that may span decades, so that the probability can be calculated from the event's past rate of occurrence. Estimating the likelihood of any event from a historical perspective depends on the amount and quality of the data (Solomon and Vallero 2016). By definition, emerging technologies lack clear and relevant historical lessons. Thus, it is not easy to determine when the potential that emerging technological exposures involve rare outcomes, e.g. cancer or loss of endangered species, i.e. low-probability, high consequence events,

which present special challenges to risk assessment and communication (Solomon and Vallero 2016). Scientific and engineering rigor are essential for rare events, as they are for any risk assessment scenario. Certainly, managing the risks presented by rare events requires many of the same fundamental communication elements of any credible risk-based decision analysis. Given the diversity of stakeholders and unconventional aspects of most rare events in which knowledge about nanotechnologies is evolving, a greater understanding and application of numerous other factors are needed, especially psychosocial and ethical factors. The potential exposure to nanomaterials is a function of a person's biological and genetic makeup, location, and activities, i.e. the so-called "exposome" of who you are, where you are and what you are doing (Wild, 2012).

For most substances, identifying potential hazards is the first step in risk assessment. Sometimes there are clues based on the physicochemical structures of analogues of similar, better known substances. All too frequently, there are little or no reliable data and information available for even crude hazard assessments for newly synthesized substances like nanomaterials. Preliminary or screening toxicity data may be available for a substance from an emerging technology if it is sufficiently similar to a known substance, but information about its potential uses and exposures are uncertain. Thus, exposure information requires both information about the substance's biological, chemical and physical properties along with human activity and use. The former information may be derived, e.g. from quantititative structure-activity relationship (QSAR) model, which is showing promise for some nanoparticles, i.e. metals (Puzyn et al. 2011). However, even if the QSAR results are acceptable for toxicity, this is only half of the risk equation, so they may not in themselves support exposure predictions, especially if the emerging technology relates to a new, understudied use or other human activity.

Risk estimates are further complicated in that it requires that the exposure estimates be combined with the inherent hazards of the substances. Regulatory programs such as REACH in Europe (Kortelainen 2015) and the U.S.'s recent efforts in exposure-based prioritization (Egeghy et al. 2011) and rapid exposure and dosimetry (Dionisio et al. 2015; Egeghy et al. 2016; Wambaugh et al. 2014; Barber et al. 2017) are a first step in identifying and categorizing the risk of substances according to potential toxicity *and* potential exposure. The risk estimate, in a sense, is akin to an index that is only as reliable as its components, in this instance the inherent toxicity, inherent physicochemical properities, and the type and likelihood of uses (Birnbaum and Jung 2011; Klaine et al. 2008; Wiesner et al. 2009). Each of these factors has uncertainty, e.g. in vitro-to-in vivo-human toxicity extrapolations (Pieters et al. 1998), QSAR uncertainties (Sahlin 2013; Sahlin et al. 2014), and known use-to-unknown use scenarios (Ernstoff et al. 2016; Beaudrie et al. 2015; Grieger et al. 2009). Thus, the uncertainty of risk predictions are propagated with each component, but added uncertainties may result when the individual components are combined, i.e. "hidden dependencies" (Hamraz et al. 2012).

Much can happen internally after a nanomaterials are absorbed. The mass at the interface between the organism and the environment, e.g. breathing zone, is merely the potential dose. Once the chemical crosses the interface it is considered applied

dose, but the toxicokinetics (ADME) begin with absorbed dose. Exposure is completed at biologically effective or target dose, i.e. when the nanomaterial or its metabolic products reach the organ/tissue that is the site of effect/outcome, e.g. the liver for a hepatotoxin brain for a neurotoxin. Any damage that results from this exposure falls in the realm of effects. For example, an exposure biomarker would show that the xenobiotic has hit the target (e.g. release of a liver enzyme), whereas an effects biomarker would show liver damage (perhaps a different liver enzyme, or the same enzyme, but at higher concentrations to indicate hepatotoxicity).

The differences between the dosimetry of nanoscale and bulk materials are not well understood. Measuring the hazard of a substance is difficult in part because the applied dose will not be the same as the absorbed and biologically effective dose, given the losses to container wall, dissolution, aggregation and other mechanisms that may be much more important for nanoscale materials, but also much more difficult to quantify at the nanoscale (Ivask et al. 2018; Sekine et al. 2015; Lead et al. 2018).

For many emerging chemicals and substances, the traditional far-field exposure scenario does not completely apply. That is, release from a source and movement within and among environmental media is often only a small fraction of the mass that comes into contact with the receptor. Most comes as a result of handling and use of products, buidling materials and articles. One means of determining whether a substance is properly regulated is whether it exceeds the level of acceptable risk. Often the criteria for deciding what constitutes too much risk are arbitrary and not necssarily based on sound science. When risk is unacceptable, governance is perceived to have failed society. Societal expectations of acceptable risk are mandated by the standards and specifications of certifying authorities. Unfortunately, these are often absent or inappropriate for new technologies that differ substantially from their conventional analogs. The conventional metrics are incorporated into health codes and regulations, zoning and building codes and regulations, design principles, canons of professional engineering and medical practice, national standard-setting bodies, and standards promulgated by international agencies (e.g. ISO, the International Standards Organization). In the United States, for example, the U.S. Environmental Protection Agency (EPA) issues guidelines, such as those for designing waste sites and rules for emissions, uses and handling of pollutants. The guidelines and rules cite other standards, e.g. mateial specifications for equipment, such as those of the National Institute of Standards and Testing (NIST) and ISO. Existing guidelines and rules can quickly become obsolete and less effective in reducing exposures in the myriad scenarios likely to arise when the new technology moves from research to application. Often, especially in the U.S., emerging technologies follow standards articulated by private groups and associations, but which may be so focused on the utility and other benefits of the technology that potential exposure and risk receives comparatively less rigorous and inadequate emphasis (Vallero 2010a).

Assessment Methodologies

Risk is generally understood to be the likelihood that an unwelcome event will occur. For public health and ecological risk, the unwelcome event is an adverse outcome in a receptor, e.g. cancer in a human population or loss of biodiversity in an ecosystem. Risk assessment is the scientific investigation into the factors that lead to a risk. An assessment may be retrospective, i.e. to see what damage has occurred; or prospective, i.e. to predict risk posed from reasonable present and future risk scenarios. Much of the risk assessment for the use of nano-scale materials is prospective. Steps needed to manage the risk are based on the risk assessment. Ideally, dispassionate and objective scientific findings must underpin decisions needed to reduce the otherwise highly likely, adverse outcomes. For example, an assessment may indicate that a particular type of nanoparticle poses a risk to human health if it were present in the water supplies. Risk management would then include the design and installation of containment structures to limit the migration of the nanoparticle from its source to the aquatic environment.

Hazards can be expressed according to the physical and chemical characteristics, as in Table 1, as well as in the ways they may affect living things. For example, Table 2 summarizes some of the expressions of biologically-based criteria of hazards. Other hazards, such as flammability, are also important to environmental engineering. However, the chief hazard in most environmental situations has been toxicity.

This chapter principally focuses on one type of hazard, i.e. toxicology. Hazard (H) is half of the risk (R) equation, with exposure (E) the second half:

$$R = f\left(H,E\right) \tag{1}$$

Risk assessment of an actual or potentially manufactured nanoscale substance, like any agent, must describe the physical, chemical and biological characterics of the hazard. The hazard is not a static agent. During its life cycle, a nanomaterial will be transformed in time and space and may act synergistically or antagonistically with abiotic and biotic components of the environments to which it is introduced. Indeed, in various parts of the life cycle the nanomaterial may, through accumulation, aggregation and other processes, may not be a nano-scale substance. For example, the metal cerium is a fuel additive as a nanoparticle,. With time, it will aggregate to a larger particles, but after combustion some of the cerium returns to the nano-scale as an ultrafine particulate matter (PM). To assess the importance of such life-cycle scenarios, the severity of the effect and the likelihood that it will occur in that scenario is calculated. This combination of the hazard and exposure particular to that scenario constitutes the risk.

The relationship between the severity and probability of a risk follows a general equation (Doblhoff-Dier et al. 2000):

Table 1 Hazards defined by the Resource Conservation and Recovery Act

Hazard type	Criteria	Physical/chemical classes in definition
Corrosivity	A substance with an ability to destroy tissue by chemical reactions.	Acids, bases, and salts of strong acids and strong bases. The waste dissolves metals, other materials, or burns the skin. Examples include rust removers, waste acid, alkaline cleaning fluids, and waste battery fluids. Corrosive wastes have a pH of <2.0 or >12.5. The U.S. EPA waste code for corrosive wastes is "D002."
Ignitability	A substance that readily oxidizes by burning.	Any substance that spontaneously combusts at 54.3 °C in air or at any temperature in water, or any strong oxidizer. Examples are paint and coating wastes, some degreasers, and other solvents. The U.S. EPA waste code for ignitable wastes is "D001."
Reactivity	A substance that can react, detonate or decompose explosively at environmental temperatures and pressures.	A reaction usually requires a strong initiator (e.g. an explosive like TNT, trinitrotoluene), confined heat (e.g. salt peter in gunpowder), or explosive reactions with water (e.g. Na). A reactive waste is unstable and can rapidly or violently react with water or other substances. Examples include wastes from cyanide-based plating operations, bleaches, waste oxidizers, and waste explosives. The U.S. EPA waste code for reactive wastes is "D003."
Toxicity	A substance that causes harm to organisms. Acutely toxic substances elicit harm soon after exposure (e.g. highly toxic pesticides causing neurological damage within hours after exposure). Chronically toxic substances elicit harm after a long period of time of exposure (e.g. carcinogens, immunosuppressants, endocrine disruptors, and chronic neurotoxins).	Toxic chemicals include pesticides, heavy metals, and mobile or volatile compounds that migrate readily, as determined by the Toxicity Characteristic Leaching Procedure (TCLP), or a "TC waste." TC wastes are designated with a waste codes "D004" through "D043."

From: Vallero 2015

$$R = f\left(S,P\right); R = S \times P \tag{2}$$

Where risk (R) is a function (f) of the severity (S) and the probability (P) of harm. The right-hand part of the equation simplifies the function as the product of severity and probability. Indeed, the units of exposure are the reciprocal of hazard units. For

Table 2 Biologically-based classification criteria for chemical substances

Criterion	Description
Bioconcentration	The process by which living organisms concentrate a chemical contaminant to levels exceeding the surrounding environmental media (e.g. water, air, soil, or sediment).
Lethal Dose (LD)	A dose of a contaminant calculated to expect a certain percentage of a population of an organism (e.g. minnow) exposed through a route other than respiration (dose units are mg [contaminant] kg^{-1} body weight). The most common metric from a bioassay is the lethal dose 50 (LD_{50}), wherein 50% of a population exposed to a contaminant is killed.
Lethal Concentration (LC)	A calculated concentration of a contaminant in the air that, when respired for four hours (i.e. exposure duration = 4 h) by a population of an organism (e.g. rat) will kill a certain percentage of that population. The most common metric from a bioassay is the lethal concentration 50 (LC_{50}), wherein 50% of a population exposed to a contaminant is killed. (Air concentration units are mg [contaminant] L^{-1} air)

Source: Wambaugh et al. 2014

instance, exposure can be expressed as mass of the pollutant per body mass per time, e.g. mg kg^{-1} d^{-1}, with the complementary hazard units being [mg kg^{-1} d^{-1}]$^{-1}$.

Articulating the hazard, i.e. the physical, chemical or biological agent of harm, is matched against the receptor's contact with that hazard, i.e. exposure. The types of receptors range in scale and complexity, for example, the exposed receptor may be:

- an individual organism, e.g. a human or other species;
- a sub-population, e.g. asthmatic children or endangered plant species in a habitat;
- an entire population, e.g. all persons in a city, nation, or the world; or,
- a macro-system, e.g. a forest ecosystem.

Hazard is an inherent trait. Thus, the hazard may occur before a waste is generated, such as a component of a manufacturing process. For example, if 1,1,1-trichloroethane (TCE) is used as solvent in a chemical processing plant, it may be hazardous to the workers because it is carcinogenic. It may also be hazardous if it finds its way to a landfill (in drums or in contaminated sawdust after a cleanup). Similarly, a genetically modified organism (GMO) has inherent properties that render it hazardous, e.g. production of exotoxins or infection of higher organisms. Thus, synthetic biological hazards are a combination of conventional hazards, e.g. the use of solvents and biological materials in the synthesis phases, and emerging hazards, e.g. the hazards introduced by a modified organism.

The second component of the risk assessment is the potential exposure to the hazard. In the previous TCE/GMO example, people can come into contact with the solvent in occupational settings and the GMO in environmental (e.g. escape) and use (e.g. drinking water). Thus, the exposure to TCE varies by activities (high for workers who use it, less for workers who may not work with TCE, but are nearby and breathe the vapors, and even less for other workers). The exposure to the GMO is zero if it is completely contained and increases with loss of containment. Also, worker exposure is commonly based on a 5-workday exposure (e.g. 8 or 10 hours),

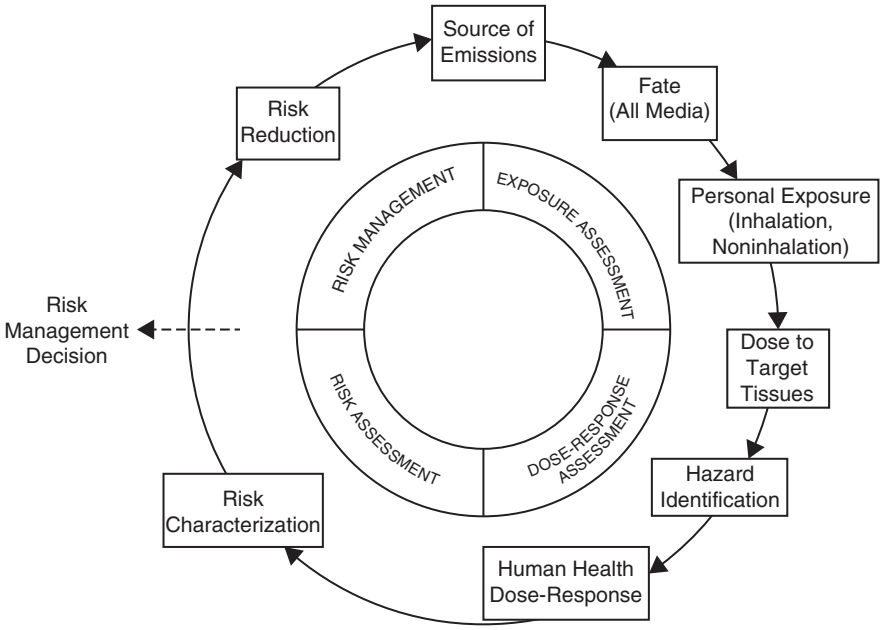


Fig. 1 Risk assessment and management paradigm as employed by environmental agencies in the United States. The inner circle includes the steps recommended by the National Research Council. The outer circle indicates the research and assessment activities that are currently used by regulatory agencies to meet these required steps. (Source: National Research Council 1983)

whereas environmental exposures, especially for chronic diseases like cancer, are based on lifetime, 24 hour per day exposures. Thus, environmental regulations are often more stringent than occupational regulations when aimed at reducing exposure to a substance.

The traditional chemical risk assessment paradigm (see Fig. 1)

is generally a step-wise process. The figure starts with the soruce of release or emission:Source
↓
Exposure
↓
Hazard
↓
Risk

This assessment draws from summaries of an agent's physicochemical properties, routes and patterns of exposure, and a review of toxic effects. The tools for hazard identification take into account the chemical structures that are associated with toxicity, metabolic and toxicokinetic properties, short-term animal and cell tests, long-term animal (*in vivo*) testing, and human studies (e.g. epidemiology, such as longitudinal and case-control studies). These comprise the core components of hazard identification, however additional hazard identification methods have been

emerging that are increasingly provide improved reliability of characterization and prediction.

Synthetic biology often involves mixtures of biological and chemical mixtures. There is no unamimity in defining "mixtures." The chemical definition distintguishes mixtures from compounds. Until relatively recently, toxicologists studied mixtures in a step-wise manner, adding substances one at a time to ascertain the response of an organism with each interation. A number of recent toxicological studies have begun to look at multi-component mixtures. From an exposure perspective, a mixture is actually a co-exposure. People and ecosystems are exposed to an array of compounds simultaneously (Kortenkamp et al. 2009). A key question is how do the individual constituents physical and chemical properties affect those of other chemical and biological constituents used during biological synthesis? The additive, synergistic and antagonistic effects must be considered.

Thus, characterizing the inherent properties of an individual constituent used in a process is the first step in risk assessment. A number of tools have emerged to assist in this characterization. Risk assessors now can apply biomarkers of genetic damage (i.e. toxicogenomics) for more immediate assessments, as well as improved structure-activity relationships (SAR), which have incrementally been quantified in terms of stereochemistry and other chemical descriptions, i.e. using QSAR and computational chemistry. There are fewer tools available for biological agents, but incorporating quantitative microbial risk assessment into a life cycle analysis (LCA) is promising (Harder et al. 2015). Health-effects research has mainly focused on early indicators of outcome, making it possible to shorten the time between exposure and a determination of a potential adverse outcome (National Research Council 2002).

To date, the greatest attention of the risk assessments has been on chemical hazards, but emerging technologies often also generate non-chemical hazards. Notably biological and infectuous wastes may be generated in certain life cycle stages that present hazards from biological agents. Increasingly, synthesis of phamaceuticals and other products involves the applications of genetic engineering and synthetic biology. Of course, biological agents range from beneficial to extremely dangerous. Indeed, the life cycle that produces a socially desired or essential drug may include exposures of workers and releases of harmful substances in various life cycle stages. For example, genetically modified microbes can be classified as (Doblhoff-Dier et al. 2000):

- Risk class 1. No adverse effect, or very unlikely to produce an adverse effect. Organisms in this class are considered to be safe.
- Risk class 2. Adverse effects are possible but are unlikely to represent a serious hazard with respect to the value to be protected. Local adverse effects are possible, which can either revert spontaneously (e.g. owing to environmental elasticity and resilience) or be controlled by available treatment or preventive measures. Spread beyond the application area is highly unlikely.

- Risk class 3. Serious adverse local effects are likely with respect to the value to be protected, but spread beyond the area of application is unlikely. Treatment and/or preventive measures are available.
- Risk class 4. Serious adverse effects are to be expected with respect to the value to be protected, both locally and outside the area of application. No treatment or preventive measures are available.

These classes indicate that even the safest microbes carry some risk and that with more uncertainty about an organism, one cannot assume it to be safe, especially for synthetic protocells and larger organisms about which little is known. The risks may not be direct, such as a change induced by the release of organism into an environment where there are no natural predictors. Thus, risk scenarios include not only the effects resulting from the intended purpose of the environmental application, but also downstream and side effects that are not part of the desired purpose. For example, the European Union (EU) requires that a synthetic biology risk assessment define the "exposure chain", i.e. the events leading to the adverse health or environmental outcome (European Union 2015).

As mentioned, the large uncertainties associated with emerging technologies call for conservative science and treating the potential hazards and exposure as risk class 4. The value of the impact could be widespread and irreversible. Also, the novelty of these technologies often means that the effectiveness and problems may differ substantially from any existing treatment or preventive measure. At best, efficacy and risk can be extrapolated from available knowledge to similar technologies, e.g. based on chemical or biological agents with similar characteristics. However, these comparisons will be conducted in similar, but yet untested environmental conditions. For example, a field study's results in one type of field could be extrapolated to a different agricultural or an environmental remediation setting. In chemical hazard identification, this is accomplished by structural activity relationships.

Prioritizing and Screening Potentially Toxic Agents

In evidence-based risk assessments, the onus is on the regulator to show that an action or agent is unsafe. This is the predominant perspective of most U.S. health and environmental regulations, i.e. it is up to the agency to stop an action, such as a new chemical being used in a product, only if sufficient information is available to show that the chemical is unsafe. However, the agency usually may require the applicant to provide such information. Chemical risk assessment is a scientific approach to answering three basic questions (U.S. Environmental Protection Agency 2004a):

1. What is the concentration of a nanoparticle or its toxic constituents in each environmental medium or compartment, e.g. in the soil, water, air, carpet, walls, etc.?
2. What is the exposure of the receptor to the NP or chemical, i.e. the amount of contact with a receptor in each medium and compartment?

3. How toxic is the NP or chemical constituent?

These three questions, respectively, address a substance's persistence, bioaccumulation and toxicity. Indeed, these three factors, when combined, determine whether a chemical compound is meets the criteria to be deemed a "PBT". This was among the first attempts of identifying environmental chemicals of concern. The EPA, for example, constructed a PBT profiler (Environmental Health Analysis Center 2012a, b) to quantify and rank chemicals. Persistence is related to the partitioning coefficients (see Table 3). The half-life ($t_{1/2}$) is a common way to express a chemical substance's persistence, i.e. the amount of time it takes to degrade one-half of the mass of a compound. This varies by media; for example, Table 3 shows the atmospheric half-lfie of several compunds.

Thus, $t_{1/2}$ is a metric of persistence, i.e. the larger the $t_{1/2}$, the more persistent the compound. Persistence is both an intrinsic and extrinsic property of a substance. It is dependent upon the molecular structure of the compound, such as the presence of aromatic rings, certain functional groups, isomeric structures, and especially the number and types of substitutions of hydrogen atoms with halogens (specifically chlorines and bromines).

Persistence is directly related to bioconcentration. Generally, the larger the $t_{1/2}$ of a substance in a compartment, the larger the bioconcentration factor (BCF). For example, the lower $t_{1/2}$ unsubstituted aliphatic compounds also often have lower BCFs, e.g. acrolein's BCF is half of the chlorinated aliphatic compound chloroform; and is 4 orders of magnitude lower than the BCF of the chlorinated aromatic compound, 2,3,7,8-tetrachlorodibenzo-*para*-dioxin.

Persistence potential also depends upon the contaminant's relationship to its media. Compound $t_{1/2}$ values are commonly reported for each compartment, so it is possible for a compound to be highly persistent in one medium, yet relatively reactive in another. Persistence varies within each compartment and depends on many factors, such as temperature, pH, intensity of sunlight, presence and diversity of microbes and chemistry (e.g. reactivity of the pollutant, as well as the concentrations of oxidizing agents, free radicals and catalysts).

Table 3 Atmospheric persistence compared to octanol–water and Henry's law coefficient

Compound	Half-life (days)	Log K_{ow}	Log K_H
Benzene	7.7	2.1	−0.6
Chloroform	360	1.97	−0.7
DDT	50	6.5	−2.8
Ethyl benzene	1.4	3.14	0.37
Formaldehyde	1.6	0.35	−5.0
Hexachlorobenzene	708	5.5	−3.5
Methyl chloride	470	0.94	−0.44
Methylene chloride	150	1.26	−0.9
PCBs	40	6.4	−1.8
1,1,1 Trichloroethane	718	2.47	0.77

Source: Toro and Hellweger 1999

The half-lives and rate constants (k) represent identically ordered decay processes, and are inversely relate to one another. For example, first-order decay can be expressed in terms of concentration versus time, concentration versus distance, and as abiotic (e.g. photochemical) and biotic (e.g. microbial) degradation rates. The relationships between $t_{1/2}$ and k can be stated mathematically. The first-order rates are:

$$k = \frac{0.693}{t_{1/2}} \tag{3}$$

And,

$$t_{1/2} = \frac{0.693}{k} \tag{4}$$

Thus, a half-life of 2 years is the same as a first-order rate constant of 0.35 year^{-1}, and a half-life of 10 years = a first-order rate constant of 0.0693 (i.e. a slower rate constant is inversely related to a longer half-life. This is an important consideration in estimating the rate at which a contaminant plume will be attenuated, and is commonly used in groundwater studies.

Note that Table 3 contains no metals or metalloids, but only organic compounds. Obviously, outside of nuclear fission or fusion, elements do not undergo degradation. For example, the degradation of the listed organic compounds does not involve destruction of any element. The carbon, hydrogen, oxygen and other atoms remain intact. However, in degradation, bonds are broken or added, elements are substituted and new compounds produced. This always involves changes in oxidation states. Thus, even though a metal is an element which will not be changed by degradation (i.e. its elemental half-life is infinite), particular metallic compounds will indeed change, just as the particular carbon compounds change in the organic degradation. Thus, even for inherently toxic compounds like mercury, $t_{1/2}$, is useful (e.g. to state the atmospheric conditions under which the methylmercury compounds degrade to elemental Hg^0 and simpler inorganic compounds).

The $t_{1/2}$ is also an important part of endogenous kinetics within an organism, including adsorption, distribution, metabolism and excretion of substances. The $t_{1/2}$ of a compound is indirectly proportional to that compound's body burden. A large $t_{1/2}$ increases the likelihood of accumulation over time. Thus, if substance A's $t_{1/2} = 1$ day, and substance B's $t_{1/2} = 100$ days, substance B would be expected to show a much larger body burden than substance A. A substance's $t_{1/2}$ also indicates which aspects of the toxicokinetics are most important. For example, <15% of inorganic Hg is absorbed by the human gastrointestinal tract (Rahola et al. 1973), whereas about 95% of methylated forms of Hg are absorbed (Aberg et al. 1969). Thus, at the same intake rate, the actual uptake is more than six times larger for methymercury than for inorganic Hg compounds.

Concentration versus time constants are known as point decay rates (k_{point}), which are derived from a single concentration value versus time plot, can be used to estimate the length of time that a plume will persist. Bulk attenuation rates (k) are derived from concentration versus distance plots, and are used to see if the contaminant plume is expanding. Thus, they are most useful for ground water contamination, but can be applied to other environmental media.

Substances that remain in the environment long after their release and deposition are more likely to continue to cause problems or to be a threat to environmental quality. The US Environmental Protection Agency considers a compound to be persistent if it has a $t_{1/2}$ in water, soil or sediment of greater than 60 days, and very persistent if the $t_{1/2}$ is greater than 180 days. In air, the compound is considered persistent if its $t_{1/2}$ is greater than two days. Some of the most notoriously toxic chemicals are also very persistent, including polychlorinated biphenyls (PCBs) and halogenated dioxins.

The worst PBT scenario is when a compound is persistent in the environment, builds up in organic tissues, and is toxic. Each factor is a function of the contaminating agent and the conditions of the environment wherein he agent resides. Recently, the United Nations Environmental Programme (UNEP) reported on the concentrations of the persistent and toxic compounds. Each region of the world was evaluated for the presence of these compounds.

The sources of PBTs are widely varied. Many are intentionally manufactured to serve some public need, such as the control of pests that destroy food and spread disease. Other PBTs are generated as unintended byproducts, such as the products of incomplete combustion. In either case, there are often measures and engineering controls available that can prevent PBT releases, rather than having to deal with them after they have found their way into the various environmental compartments. One of the principal reasons for the concern about the plethora of organic chemicals and heavy metals in the environment has been the connection between exposures to these substances and cancer and other chronic diseases. Intrinsic properties of compounds render them more or less toxic. In addition, physical and chemical properties determine whether the compounds will resist degradation and persist for long time periods and build up in organisms.

The concept of persistence elucidates the notion of tradeoffs that are frequently needed as part of many responses to environmental insults. It also underscores the importance of sound science and reliable data. For example, the pesticide DDT [1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane ($C_{14}H_9Cl_5$)] is relatively insoluble in water (1.2–5.5 mg L^{-1} at 25 °C) and is not very volatile (vapor pressure: 0.02×10^{-5} mmHg at 25 °C (UNEP 2003). The water solubility and vapor pressures alone may indicate that people and wildlife are not likely to be exposed in the air or water. However, the compound is highly persistent in soils, with a $t_{1/2}$ of about 1.1–3.4 years, so it may still end up in drinking water in the form of suspended particles or in the air sorbed to fine and ultrafine particles. DDT also exhibits high bioconcentration factors (BCF), i.e. approximately 50,000 for fish and 500,000 for bivalves. This indicates that once organisms become exposed, they tend to increase

body burdens of DDT over their lifetimes. In the environment, the parent DDT is metabolized mainly to DDD and DDE.[1]

There is a noteworthy exception to the continuous increase in body burden over an organism's lifetime. During gestation, there can be *in utero,* cross-placental transfer of lipophilic compounds from the fat reserves of the mother to the baby. The initial peaks in both the male and the female whale represents contaminant offloading via the mother's milk. Since PCBs and pesticides are typically found in fat (i.e. they are highly lipophilic) and because milk is the means by which the baby receives the necessary fat for nutrition, the neonate receives large doses of these contaminants during the weaning stage of development. When rate of weaning diminishes, the uptake by the calf declines. This is a detoxification or depuration stage during which more mass of the contaminant is excreted than is absorbed. After this, for the rest of the male's life, the persistent compound body burden increases; whereas for the birthing stage of the female, there is an oscillation between contaminant offloading to the calves and accumulation periods.

Many nations take what is known as the precautionary approach. Regulators in these nations will deny any application for product or process that may lead to severe and potentially irreversible harm unless the applicant can provide sufficient information showing that the action or product is safe (Persson 2016; Singh 2016; Turvey et al. 2005; Harremoës et al. 2001; Science and Environmental Health Network 1998). Unlike traditional risk assessments, which assume the regulatory agency holds the burden of proof that something is harmless, the precautionary onus assumes that the burden of proof is entirely on those who anticipate the new action and if "reasonable suspicion" arises of a severe and potentially irreversible outcome, the action as proposed should be denied. This requires an objective, well-structured, comprehensive analysis of alternatives to provide a needed service, including a "no-action" if any of the alternatives are worse than doing nothing new.

Other regulatory structures are employed around the world. The REACH rule in Europe is an example of the precautionary principle in practice, but tends to be driven by hazard assessments, with less but a growing emphasis on exposure (Gustavsson et al. 2017). Other countries, especially developing nations, have yet to prioritize chemicals with either approach, or are just beginning (Mansour et al. 2016; Diamond et al. 2015).

Increasingly, evidence-based risk assessments are being augmented or even supplanted by precaution, especially if the decision involves what a specific agency deems to be a reasonable likelihood that an adverse effect is severe and irreversible (United Nations Environment Programme 1992). The precautionary approach also calls for systems thinking and sustainable solutions. Whatever the approach, new screening models and tools are needed, including multi-criteria decision analysis (MCDA), which allows for the consideration of numerous variables, from various information sources. New ways to communicate risk and reliability include expert

[1]The two principal isomers of DDD are: *p,p'*-2,2-bis(4-chlorophenyl)-1,1-dichloroethane; and *o,p'*-1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane. The principal isomer of DDE is *p,p'*-1,1'-(2,2-dichloroethenylidene)-bis[4-chlorobenzene].

elicitation (Wood et al. 2020), i.e. gathering insights from a swath of the scientific community on newly emerging or otherwise poorly understood challenges to air quality.

Among the important gaps in data and tools needed for risk-based and precaution-based decision making is more reliable means of estimating and predicting exposures to stressors. For example, human health characterization factors (CFs) in LCAs have benefited from improved hazard, especially toxicity, information (Schulte et al. 2013). Given that health risk is a function of hazard and exposure, both traditional risk assessment and LCA's human health CF must be based on reliable exposure predictions (Vallero 2016). For instance, in early life stages that, when used, could result in the formation and release of pollutants at some later stage, e.g. eliminating wastes before the reach the household or changing chemical synthesis or product manufacturing approaches in early stages that prevent a future problems (Gauthier et al. 2015).

Emerging Focus on Near-Field Exposure Assessments

Chemical risk assessment was codified for federal agencies in 1983 by the National Research Council (NRC) of the National Academy of Sciences (National Research Council 1983). Air was among the media addressed. Air pollution engineers and managers may be tempted to perceive their roles as limited to laws and rules specifically targeted to address air quality. In the U.S., the main statutory authority rests with the Clean Air Act and its amendments, which predominantly considers pollutants from a "far-field" perspective. That is, a substance is released, and exposure occurs after some time and within some space beyond the source. Thereafter, risk is calculated based on the toxicity of substances and the likely exposure to those substances. However, these laws and rules are not the only ones that drive risk. Recently, the Toxic Substance Control Act and other laws have authorized greater attention to "near-field" exposure scenarios, i.e. exposure occurs when a person uses a product or engages in some other activity within a home or other microenvironment.

Several NRC reports subsequent to the 1983 red book have since provided guidance on ways to assess risks of chemicals, including reports that highlighted the disparity between the rate of deployment of new anthropogenic chemicals and assessment of their potential risks to public health (National Research Council 2007, 2009a). Central to these evolving recommendations has been to replace the current practice of extensive animal-based characterization of chemical hazard, dose–response relationships, and extrapolation to human health with high-throughput *in vitro* tests, *in silico* models and evaluations of efficacy at the human population level. In addition, human and ecosystem risk assessments require reliable approaches for exposure to these chemicals. Noting that risk is a function of both hazard and exposure, the NRC added recommendations for advancing the risk-based science that underpins environmental and human health decision making (National Research Council 2012). Among these recommendations is to

introduction of credible ways to screen and to prioritize chemical substances before these chemicals become ingredients in products and components in articles that reach the marketplace. Such an exposure-based prioritization approach will depend on the high throughput (HTP) and other tools that are not only rapidly deployed, but which are scientifically sound.

In the U.S., the Clean Air Act and its amendments have driven the selection of air pollutants of concern, which fall into two categories, i.e. "criteria" and "hazardous" air pollutants. The criteria pollutants are lead (Pb), tropospheric ozone (O_3), carbon monoxide (CO), nitrogen oxides (NO_x), sulfur dioxide (SO_2), PM with aerodynamic diameters of 10 microns or less (PM_{10}), and PM with diameters of 2.5 microns or less ($PM_{2.5}$). The 187 hazardous pollutants include organic and inorganic compounds, including compounds of mercury (Hg), hydrochloric acid (HCl) and other acid gases, heavy metals such as nickel and cadmium, and hazardous organic compounds such as benzene, formaldehyde, and acetaldehyde are included among these HAPs.

Although this may appear to be a large number of compounds regulated under air pollution laws, it is dwarfed by other regulations, especially those regulated under the Toxic Substances Control Act (TSCA) and its amendments, Unlike the Clean Air Act, which regulates emissions, TSCA considers the potential risks from potential exposure to ingredients in yet-to-be-released products and estimated risks for products already in use. If the risks are unacceptable, new products may not be released as formulated or the uses will be strictly limited to applications that meet minimum risk standards. For products already in the marketplace, the risks are periodically reviewed, although often in a less stringent manner.

Another product-related development in recent years is the growth in the importance of screening and prioritizing chemicals for possible harm and exposure prior to their appearance in the marketplace. For example, research suggests a link between exposure to certain chemicals and damage to the endocrine system humans and wildlife. In the U.S., the Endocrine Disruptor Screening Program focuses on methods and procedures to detect and to characterize the endocrine activity of pesticides and other chemicals (U.S. Environmental Protection Agency 2017a).

TSCA gives the EPA the authority to track thousands of industrial chemicals currently produced or imported into the U.S. This is accomplished through screen of the chemicals and requiring that reporting and testing be done for any substance that presents a hazard to human health or the environment. If chemical poses a potential or actual risk that is unreasonable, the EPA may ban the manufacture and import of that chemical.

Governments in North America, Europe and Asia track thousands of new chemicals being developed by industries each year, if those chemicals have either unknown or dangerous characteristics. This information is used to determine the type of control that would be needed to protect human health and the environment from these chemicals. Manufacturers and importers of chemical substances first submit information about chemical substances already on the market during an initial inventory. Since the initial inventory was published, commercial manufacturers or importers of substances not on the inventory have been subsequently required to submit

notices to the EPA, which has developed guidance about how to identify chemical substances to assign a unique and unambiguous description of each substance for the inventory. The categories include:

- Polymeric Substances;
- Certain Chemical Substances Containing Varying Carbon Chain
- Products Containing Two or More Substances, Formulated and Statutory Mixtures; and
- Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

Historically, air pollution has mainly been concerned with so-called "far-field" exposure scenarios, i.e. those where a pollutant is released and finds its way to the receptor. Indoor air pollution is a "near-field" exposure scenario in which pollutants are either generated indoors or penetrated from far-field pollutants. More recently, exposure from product use is increasingly the focus of risk assessors and researchers. As evidence, the European Commission and individual nations are strengthening their methods for screening and prioritizing chemicals based on precautions before and after these substances enter the marketplace. The U.S. Congress recently amended TSCA to prioritize and evaluate the risks of existing chemical substances. The law contains deadlines and minimum requirements for the number of chemicals that must undergo risk evaluation and lays out a process and the criteria by which prioritization and risk evaluation must be conducted. A chemical designated as low-priority indicates a risk evaluation is not warranted at that time. Final designation of a chemical or chemical category as a high-priority immediately initiates the risk evaluation process (Final Rule 2018). TSCA requires that high-priority chemicals undergo risk evaluation to determine whether a chemical presents an unreasonable risk of injury to health or the environment, without consideration of costs or other non-risk factors, including an unreasonable risk to a potentially exposed or susceptible subpopulation.

Meanwhile, systems sciences have been rapidly advancing across the disciplines, e.g. systems biology and systems chemistry. The systems approach has been employed in research and development, as well as in practice, e.g. systems engineering and systems medicine (Jain 2017; Jain et al. 2011; Sample and Charles 2012). In recent decades, engineering and biomedicine have relied less on reductionist thinking, and increasingly call for translational science from one discipline to another (Chan et al. 2016; Heath 2015). This requires that biological, chemical, and physical principles be meshed with the social sciences to explain why and how systems, including the human body and ecosystems, respond to stress. Engineers and physicians, of course, have long recognized that the real-world cannot be reduced to the sum of its parts, and that failure analysis and disease diagnosis and treatment almost always has included "black boxes" (Modeling 2018). This is the quarry of systems thinking, i.e. explaining how these black boxes work and apply these lessons to real-world problems, including environmental quality.

Of the thousands of chemicals in the marketplace, many will be present in nanotechnology industrial and use scenarios. Some chemicals are nearly ubiquitous.

They can be found in myriad manufacturing processes, consumer products and in the environment (Nanotechnology Industries Association 2019; European Chemicals Agency (ECHA) 2014). Some chemical compounds are inherently toxic, some so extremely toxic that even extremely low concentrations are deemed to be an unacceptable risk. Some of these are particularly persistent and bioaccumulate in the environment and the food chain (Environmental Health Analysis Center 2012a, b). When a chemical compound is both harmful *and* likely to reach people and ecosystems, they present special challenges.

Prioritizing these chemicals based on the harm they may cause is now an international concern (Egeghy et al. 2011; Dix et al. 2007; Gangwal et al. 2012; Judson et al. 2010; Wambaugh et al. 2013). However, such prioritization is complex in that many chemicals may be toxic parent compounds that degrade into other compounds. Some of these may be even more toxic, persistent and/or biodegradable than the parent compounds (Mackay and Fraser 2000). Even parent compounds that are not so toxic may degrade into toxic compounds. It must also consider the likelihood of contact with receptors. For example, a chemical used in an industrial process may be relatively safe within an industrial life stage if workers are wearing proper personal protection equipment, but in downstream life cycle stages may become problematic. During their residence time in solid waste, wastewater or other environmental media may allow them to combine and react with other substances within these substrates to become more hazardous. In nature and engineered bioreactors, e.g. landfills, microbial populations help to degrade organic compounds (Boonyaroj et al. 2017; Long et al. 2009; Muñoz et al. 2007; U.S. Environmental Protection Agency 2007), and may also render metallic and inorganic compounds less toxic and less mobile by changing oxidation states (Ehrlich 2017). However, these processes can also lead to unwanted products, e.g. increasingly toxic degradation products, i.e. bioactivation (Sims and Steevens 2008; Williams and Park 2003). Nanoparticles can be affected by these processes both physically and biologically. They may aggregate in porous media and water and they may be sorbed onto sediment and soil particles, where the NP constituents are transformed.

Most chemical screening, until relatively recently, has been based on the inherent properties of chemical. The screens were built from historical data from animal and epidemiological studies, often based on pure doses. Recently, screening has been based on both hazard, especially toxicity, and exposure information. For example, exposure prioritization can complement and/or be integrated into decision tools, such as EPA's Chemistry Dashboard (Karmaus et al. 2016), which includes individual chemical structures for over 700,000 compounds, and combines bioassay screening data, exposure modes, and product categories. Screening tools can be beneficial in identifying analytics associated with data-poor and emerging substance, e.g. nanomaterials, by showing rankings of chemicals based on hazard and exposure potentials (Wood et al. 2020). Such screening tools can be also support the evaluation of a hypothetical portfolio of products (e.g., cleaning products and cosmetics) for various life stages of a product. A portfolio of products and an accompanying set of their chemical ingredients can allow decision makers to rank products

according to potential risk, including the likelihood of the formation and transformation of pollutants (Anastas and Lankey 2000).

Exposure Probability Assessments

Like the hazard identification process for chemicals, a natural or synthetic microbe is classified according to inherent properties. It is in the next stage that environmental conditions are taken into account; characterizing different responses to dose in different populations. Both the hazard identification and dose-response information are based on research that is used in the risk analysis. For microbes, the highest score for any one determines the overall risk class for environmental application. In addition, the exposure estimate is the sum of all the exposures, i.e. the evaluation of the likelihood of the occurrence if each potentially adverse outcome (European Union 2015). This guidance would be particularly useful to nanobiotechnology risk and exposure assessments.

The factors leading to the exposure probability include the release, replication, dispersion and ultimate contact with the microbe and other contaminants produced during and after the synthesis. The release may be intentional, e.g. use of the product during medical, veterinary, agricultural and consumer activities, and unintentional, e.g. during laboratory studies and manufacturing.

Managing exposures to nano-scale substance (and any pollutant for that matter) must consider protecting the most vulnerable members of society, especially pregnant women and their yet-to-be-born infants, neonates, and immunocompromised subpopulations. Also, the exposure protections vary by threat. For example, adolescents may be particulary vulnerable to hormonally active agents, including many pesticides.

In the United States, ecological exposure and risk assessment paradigms have differed from those applied to human health risk. The ecological risk assessment framework (see Fig. 2) is based mainly on characterizing exposure and ecological effects. Both exposure and effects are considered during problem formulation (US Environmental Protection Agency 1992).

Interestingly, the ecological risk framework is driving current thinking in human risk assessment. The process shown in the inner circle of Fig. 1 does not target the technical analysis of risk so much as it provides coherence and connections between risk assessment and risk management.. When scientific assessment and management are carried out simultanously, decision making could be influenced by the need for immediacy, convenience or other political and financial motivations. The advantage of an arms-length, bifurcated approach is that decisions and management of risks are based on a rational and scientifically credible assessement (Loehr et al. 1992; Ruckelshaus 1983).

In both human health and ecological assessments, the final goal is “characterization”, i.e. integrating the “quantitative and qualitative elements of risk analysis, and of the scientific uncertainties in it” (National Research Council 2009b). The

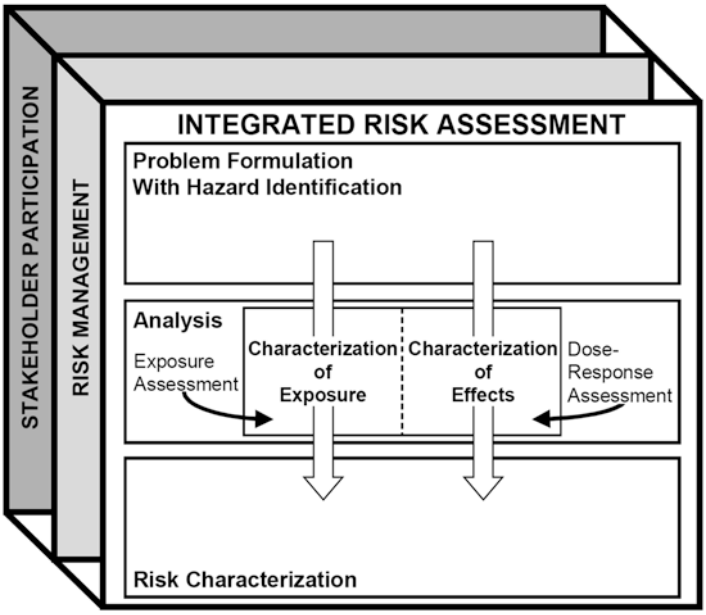


Fig. 2 Framework for integrated human health and ecological risk assessment. (Sources: US Environmental Protection Agency 1992; World Health Organization 2000)

problem formulation step in the ecological framework has the advantage of providing an analytic-deliberative process early on, since it combines sound science with input from various stakeholders inside and outside of the scientific community.

The ecological risk framework calls for the characterization of ecological effects instead of hazard identification used in human health risk assessments. The term "hazard" has been used in chemical risk assessments to connote either intrinsic effects of a stressor or a margin of safety by comparing a health effect with an estimate of exposure concentration. Thus, the term becomes ambiguous when applied to non-chemical agents, such as microbial hazaards. Specific scientific investigations will often be needed to augment exisitng assessment methods and data, especially when adverse outcomes may be substantial and small changes may lead to very different functions and behaviors from unknown and insufficiently known chemicals or microbes. For example, a genetically modified microbe (GMM) may have only been used in highly controlled experiments with little or no information about how it would behave inside of an organism. Often, the proponents of a product will have done substantial research on the benefits and operational aspects of the chemical constituents, but the regulatory agencies and the public may call for more and better information about unintended and yet-to-be-understood consequences and side effects (Doblhoff-Dier et al. 2000).

Even when much is known, there often remain large knowledge gaps when trying to estimate environmental impacts. The bacteriusm *Bacillus thuringiensis,* for instance, has been applied for several decades as a biological alternative to some

chemical pesticides. It has been quite effective when sprayed onto cornfields to eliminate the European corn borer. The current state of knowledge indicates that this bacterium is not specific in the organisms that it targets. What if in the process, *B. thuringiensis* also kills honey bees? Obviously, this would be a side effect that would not be tolerable from either an ecological or agricultural perspective (the same corn crop being protected from the borer needs the pollinators). Furthermore, physical, chemical and biological factors can influence these effects, e.g. type of application of Bt can influence the amount of drift toward non-target species. Downstream effects can be even more difficult to predict than side effects, since they not only occur within variable space, but also in variable time regimes. For example, exposure potential can arise from both the application method and from the build up of toxic materials and gene flow following the use of a GMM.

Mapping Exposure Metrics onto Toxicity Values

The predicted no-effect concentraton (PNEC) is a key toxicity metric employed for nanoparticles. This is the concentration of a substance below which no effect is expected. In this sense, it is a forward-looking threshold analogous to the retrospective no adverse effect concentration (NOAEC). PNEC values are derived from acute toxicity data, exposure to bacteria, e.g. *bacillis subtilis* and *escherichia coli*, to a nanoparticle to determine threshold concentrations for each species.

The PNEC and NOAEC for a substance are directly proportional because the PNEC can be derived by dividing the NOAEC by a series of assessment factors to address safety and uncertainty. Thus, if uncertainties are high, very large safety factors are used, so that the PNEC is much smaller than the NOAEC for a substance. Unfortunately, assessment factors can be arbitrary and PNECs for many substances avre not quantified, limiting their use to well-studied, data-rich substances (Jin et al. 2012). This excludes most nanoparticles.

Often the PNEC is compared to existing concentrations in one or more environmental media or to the predicted environmental concentration (PEC). Ideally, the risk quotient $\frac{\text{PNEC}}{PEC}$ should be much less than 1.

From a high-throughput, high-tier screening perspective, the first order problem occurs when the likelihood of exposure *and* the likelihood of hazard coexist. No matter the steepness of the hazard curve of substance, it will only elicit risk when it comes into contact with the receptor. For example, in the EPA's risk-based chemical prioritization efforts, the substances of greatest concern are those with high potential toxicity and high potential exposure profiles. Indeed, even when toxicity remains the same, those substances with higher exposure potential will lead to higher risk (see far-right of Fig. 3). The listing of contaminants of concern by this approach will differ from one based solely on toxicity, since even moderately toxic substances can have high risk potentials. Similarly, when hazard concentrations (e.g. greater than

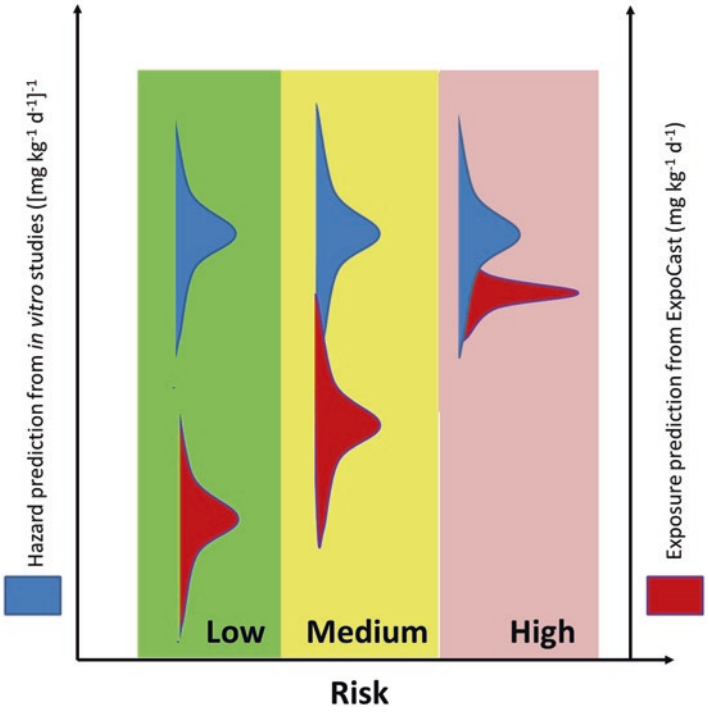


Fig. 3 Potential risk extrapolated from constant toxicity and increasing exposure potential. (Based on: Judson et al. 2011)

PNEC) and exposure potential intersect, there is greatest concern, since populaitons are being exposed at concentrations most likely to lead to adverse outcomes.

Similarly, exposure calculations are linked to risk estimates by metrics based on toxicity thresholds that are modified by uncertainty factors (UFs). The uncertainties are largely due to the differences between results found in animal testing and expected outcomes in human population. For environmental risk analyses and assessments, the safe level is expressed in the RfD, or in air the RfC. This is the dose or concentration below which regulatory agencies do not expect a specific unacceptable outcome. Thus, all the uncertainty factors adjust the actual measured levels of no effect (i.e. the threshold values, e.g. NOAELs and LOAELs) in the direction of a zero concentration. This is calculated as:

$$RfC = \frac{\text{Threshold}}{UF_{\text{inter}} + UF_{\text{intra}} + UF_{\text{other}}} \tag{5}$$

The first of the three types of uncertainty are those resulting from the difference in the species tested and that of humans (UF_{inter}). The UF_{intra} results from the differing sensitivities of human subpopulations when exposed to a substance than those of the

general human population. The UF_{other} occurs because available data and science are lacking, including the uncertainty introduced when, the data may indicate a lowest observed adverse effect concentration (LOAEC), but not a NOAEC, i.e. the LOAEC-to-NOAEC (U_L). That is, data show a dose at which an effect is observed, but the "no effect" threshold cannot be delineated. In a hypothetical example, let us calculate the RfC by using the that the animal data from Landsiedel et al. (Landsiedel et al. 2010) for a titanium dioxide (TiO_2) NP show no NOAEC but have a LOAEC of 2 mg m^{-3} and a silicate (SiO_2) NP has a NOAEC of 10 mg m^{-3}. Using the EPA's (Benson et al. 2002) most protective recommended U_L of 10 and assuming the remaining UF_{inter} = 10 for and UF_{intra} = 100 for both NPs, then:

$$RfC - TiO_2 = \frac{2}{10 \times 100 \times 10} = 0.0002 \text{mgm}^3$$

$$RfC - SiO_2 = \frac{2}{10 \times 100} = 0.01 \text{mgm}^3$$

Thus, although the thresholds may indicate that the toxicity is similar for the two NPs, given the lack of data to delineate a NOAEC, the TiO_2 NP requires a 50 times larger factor of safety.

Since the UFs are in the denominator, the greater the uncertainties, the closer the safe level (i.e. the RfC) is to zero, i.e. the threshold is divided by these factors. The UFs are usually multiples of 10, although the UF_{other} can range from 2 to 10.

Children and child-bearing age women are obvious sensitive subpopulations. As evidence, the Food Quality Protection Act (FQPA) now includes what is known as the "10X" rule. This rule requires that the RfC for products that contain pesticides, for example, must assume that infants, children and females between the ages of 13 and 50 years old are exposed at a factor of 10 higher than mean exposure calculations. This factor is included in the RfC denominator along with the other three UF values. The RfC that includes the UFs and the 10X protection is known as the population adjusted dose (PAD). A risk estimate that is less than 100% of the acute or chronic PAD does not exceed the Agency's risk concern.

An example of the use of a reference dose or concentration as a factor of safety can be demonstrated by the U.S. Environmental Protection Agency's decision making regarding the re-registration of the organophosphate pesticide, chlorpyrifos. The acute dietary scenario had a NOAEL of 0.5 mg kg^{-1} d^{-1} and the three UF values equaled 100. Thus, the acute RfD = 5×10^{-3} mg kg^{-1} d^{-1} but the more protective acute PAD 5×10^{-4} mg kg^{-1} d^{-1}. The chronic dietary scenario is even more protective, since the exposure is long-term. The chronic NOAEL was found to be 0.03 mg kg^{-1} d^{-1}. Thus, the chronic RfD for chlorpyrifos = 3×10^{-4} mg kg^{-1} d^{-1} and the more protective acute PAD 5×10^{-5} mg kg^{-1} d^{-1}. Therefore, had the NOAEL threshold been used alone without the safety adjustment of the RfD, the allowable exposure would have been three orders of magnitude higher (Wiesner et al. 2009).

Routes and Pathways of Exposure

The typical routes of exposure are inhalation, ingestion, and dermal. Humans and other organisms can come into contact with nanomaterials through these and other routes, including nasal and occular. Whereas exposure to manufactured NPs and other nanomaterials can be by many routes, inhalation is almost always involved in human exposues. Thus, given limited space, this section focuses on human, rather than ecosystem expsoures, this discussion illustrates NP exposure via human inhalation. In addition, it is important to keep in mind that nanoscale exposure scenarios also include gas-phase pollutants and that all pollutants released in nanotechnology can reach the receptor via all exposure pathways and routes. In some exposure scenarios, these may be larger problems than those presented by particulate-phase pollutant inhalation exposures.

Nanomaterial aerosols may consist of living matter, e.g. a modified cell, genetic material or a whole genetically modified microbe (GMM), or abiotic substances, e.g. metal oxides and organic compounds in an engineered nanoparticle (NP). For GMMs, the EU (European Union 2015) has identied the potential for aerosol generation to be among the key factors in calculating the probability of exposure, along with the scale of the activity, concentration and volume (cultures, supernatants, etc.), setting and type of activity (e.g. in vivo or in vitro).

The exposure to a synthetic biological aerosol can illustrate the similarities and differences in nanomaterial dosimetry. Another key characteristics of nanoscale aerosols is their source. In particular, NP are engineered for a specific purpose. As such they are likely to be more homogeneous in size, morphology, coatings (Warheit 2004) and oxidative potential (Okabe et al. 1973) compared to ultrafine PM, albeit both have the same size cutoff, i.e. <100 nm aerodynamic diameter. In addition, there is a time function associated with ultrafines in the atmopshere, i.e. after emission they begin to aggregate in to larger diameter particles and configurations. In spite of these differences, much of what has been learned from ultrafines can be extrapolated and applied to NP exposure and effect in at least four ways (Stone et al. 2017):

1. The large body of literature linking adverse human health effects to PM exposure, and more recently ultrafines, dwarfs the initial studies that have suggested an association between exposure to nanomaterials and human health, but with relatively few clinical or epidemiology data are currently available.
2. Data from PM epidemiology and mechanistic research provides and causation base on which to develop hypotheses for NP and nanomaterial potential modes of action.
3. Conversely, NP and nanoscale research can improve the understanding of mechanisms that lead to adverse outcomes for ultrafine exposure.
4. Characterization of ultrafine factors that lead to toxicity and exposure can be applied to NP and nanomaterial toxicity and exposure. Factors include dispersion, size, agglomeration, and morphology. Such characterizations can improve

mutually useful sampling and analytical methods, e.g. standard operating procedures for filter selection and microscopy.

The Inhalation Route

The human respiratory tract can be divided into three regions, i.e. the extrathoracic, tracheobronchial, and alveolar. The extrathoracic region consists of airways within the head, i.e. nasal and oral passages, through the larynx and represents the areas through which inhaled air first passes. From there, the air enters the tracheobronchial region at the trachea. From the level of the trachea, the conducting airways then undergo dichotomous branching for a number of generations. The terminal bronchiole is the most peripheral of the distal conducting airways and leads to alveolar region where gas-exchange occurs in a complex of respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. Except for the trachea and parts of the mainstem bronchi, the airways are surrounded by parenchymal tissue composed mainly of the alveolated structures and blood and lymphatic vessels. Incidentally, the respiratory tract regions are made up of various cell types and the distribution of cells that line the airway surfaces have different anatomical qualities in the three regions (U.S. Environmental Protection Agency 2004b).

The first exposure characterization of a particle is its size and shape. The behavior of a particle of any size in the lung depends on the aerodynamic characteristics of particles in flow streams. In contrast, the major factor for gases is the solubility of the gaseous molecules in the linings of the different regions of the respiratory system (see Fig. 4). Thus, the size and morphological features of NPs and ultrafine PM will affect rates of dissolution and desorption in the lung surfactant. Given the size of nanoparticles, they may behave at times as an aerosol and other times as a gas.

The aerodynamic properties of particles are determined not only by size, but also by their shape and density. The behavior of a chain agglomeraion, nannotube or fiber may also be dependent on its orientation to the direction of flow. The deposition of particles in different regions of the respiratory system depends on their size. The nasal openings permit very large dust particles to enter the nasal region, along with much finer airborne PM. Incidently, air pollution scientists and engineers call PM with aerodynamic diameters of less than 100 nm, i.e. the upper size range of nanoparticles, “ultrafine PM”.

Larger particles are deposited in the nasal region by impaction on the hairs of the nose or at the bends of the nasal passages (Fig. 5). Smaller particles pass through the nasal region and are deposited in the tracheobronchial and pulmonary regions. Particles are removed by impacts with the walls of the bronchi when they are unable to follow the gaseous streamline flow through subsequent bifurcations of the bronchial tree. As the airflow decreases near the terminal bronchi, the smallest particles are removed by Brownian motion, which pushes them to the alveolar membrane (Vallero 2014).

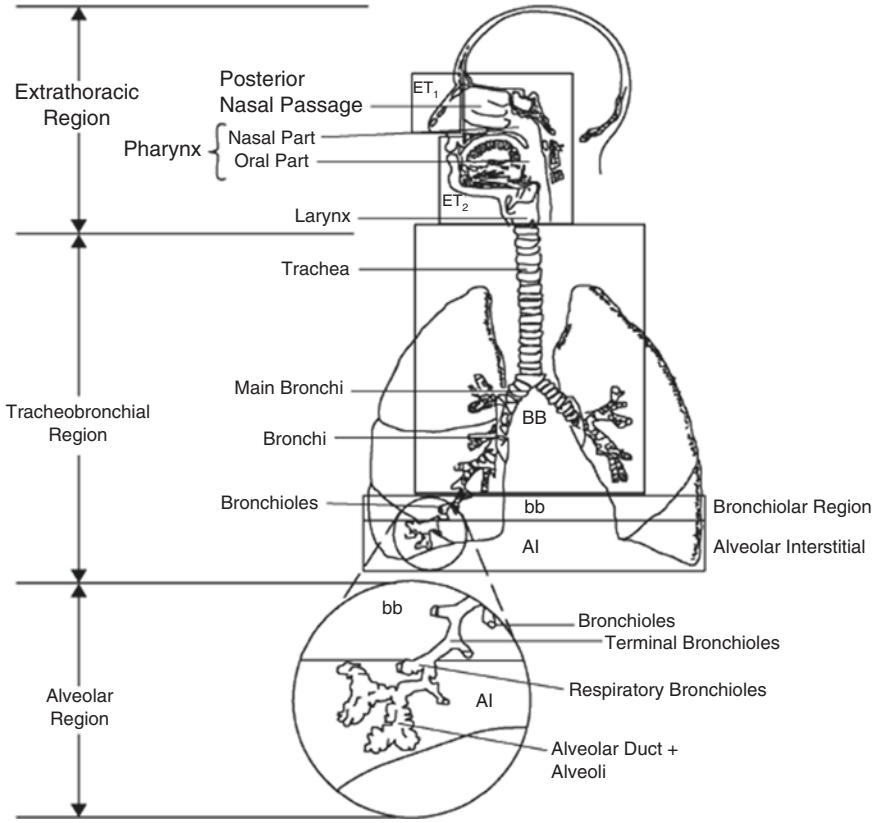


Fig. 4 Anatomy of the human respiratory tract. (Source: U.S. Environmental Protection Agency 2004b)

The respiratory system has several mechanisms for removing deposited aerosols. The walls of the nasal and tracheobronchial regions are coated with a mucous fluid. The tracheobronchial walls have fiber cilia which sweep the mucous fluid upward, transporting particles to the top of the trachea, where they are swallowed. This mechanism is often referred to as the mucociliary escalator. In the pulmonary region of the respiratory system, foreign particles can move across the epithelial lining of the alveolar sac to the lymph or blood systems, or they may be engulfed by scavenger cells called alveolar macrophages. The macrophages can move to the mucociliary escalator for removal. For gases, solubility controls removal from the airstream. Highly soluble gases such as SO_2 are absorbed in the upper airways, whereas less soluble gases such as NO_2 and ozone (O_3) may penetrate to the pulmonary region. Irritant gases are thought to stimulate neuro-receptors in the respiratory walls and cause a variety of responses, including sneezing, coughing, bronchoconstriction, and rapid, shallow breathing. The dissolved gas may be eliminated by biochemical processes or may diffuse to the circulatory system (Vallero 2008).

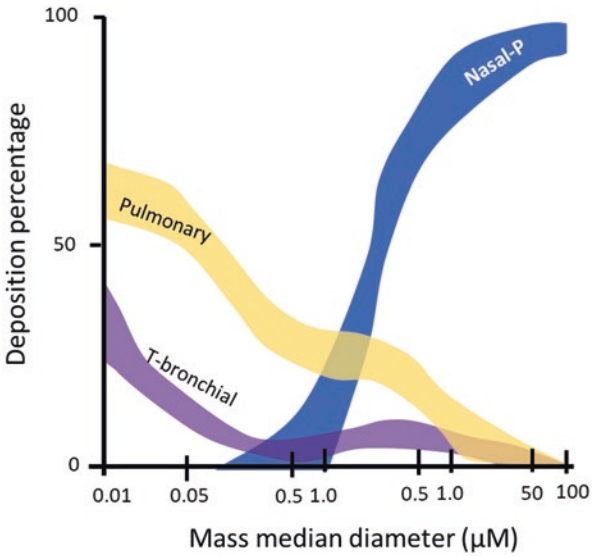


Fig. 5 Particle deposition as a function of particle diameter in various regions of the lung, from nanoparticles (10–100 nm) to coarse particles (>10 μm). The nasopharyngeal region consists of the nose and throat; the tracheobronchial (T-bronchial) region consists of the windpipe and large airways; and the pulmonary region consists of the small bronchi and the alveolar sacs. (Source of data: International Commission on Radiological Protection Task Force on Lung Dynamics, Task Group on Lung Dynamics 1966)

Moderately sized particles (1–5 μm) are more likely to deposit in the central and peripheral airways and in the alveoli but are often scavenged by macrophages. Particles with an aerodynamic diameter less than 1 μm remain suspended in air and are generally exhaled. Thus, NPs are likely to penetrate quite deeply.

Inhaled NPs may alter the lung tissue, changing the respiratory system either directly (e.g. airway inflammation) or indirectly (e.g. by altering its immune response). Susceptibility to air pollutants differs among individuals, as exemplified by several diseases and conditions (e.g., asthma), but the fluid dynamics are the same, i.e. disruption of the movement of air into the lungs to provide oxygen.

Respiratory Fluid Dynamics

The motion of air and gases in the respiratory system follow fundamental fluid dynamic principles (Issacs et al. 2012). The motion of these fluids is governed by the conservation of mass (continuity) equation and conservation of momentum (Navier-Stokes) equation. To this point, the mechansims have been explained descrptively; however fluid principles that apply to NP and nanomaterial exposure and dose are best explained quantitively. The reader is reminded that the air and

inhalation are merely one exposure pathway and route for NPs, respectively. Similar quantifications must be made for other pathways and routes, such as skin penetration and diffusion partitioning for the dermal route (Larese et al. 2009) and digestive, absorptive, distributive, metabolic, secretory, elimination and protective mechansims for the ingestion route (Bergin and Witzmann 2013).

Under most conditions, the flow of air in the respiratory airways is assumed to be incompressible. For incompressible flow, the continuity equation is expressed as[2]:

$$\nabla \cdot V = 0 \tag{6}$$

And, the continuity equation is:

$$\rho\left[\frac{\partial V}{\partial t} + (V \cdot \nabla)V\right] = \rho f - \nabla p + \mu \nabla^2 V \tag{7}$$

Where, ∇ is a gradient operator; ∇^2 is a Laplacian operator; V is velocity; ρ is fluid density; μ is absolute fluid viscosity; p is the hydrodynamic density; and f is a volumetric force that is applied externally, e.g. gravity.

For cylindrical profiles like bronchi, the gradient operator ∇ can be expressed in cylindrical coordinates:

$$\frac{\partial}{\partial r} + \frac{1}{r}\frac{\partial}{\partial \theta_\theta} + \frac{\partial}{\partial z} \tag{8}$$

Thus, the continuity equation can also be expressed cylindrically:

$$\frac{1}{r}\frac{\partial}{\partial r}(rV_r) + \frac{1}{r}\frac{\partial}{\partial \theta}V_\theta + \frac{\partial}{\partial z}V_z = 0 \tag{9}$$

Where, ∇_r, ∇_θ and ∇_z are the components of the fluid velocity, which are depicted in Fig. 6, i.e. radial (r), circumferential (θ) and axial (z) directions, respectively. Thus, the momentum equations in these directions can be expressed as:

$$\frac{\partial V_r}{\partial t} + (\boldsymbol{V} \cdot \nabla)V_r - \frac{1}{r}V_\theta^2 = -\frac{1}{\rho}\frac{\partial p}{\partial r} + f_r + \frac{\mu}{\rho}\left(\nabla^2 V_r - \frac{V_r}{r^2} - \frac{2}{r^2}\frac{\partial V_\theta}{\partial \theta}\right) \tag{10}$$

$$\frac{\partial V_\theta}{\partial t} + (\boldsymbol{V} \cdot \nabla)V_\theta + \frac{V_r V_\theta}{r} = -\frac{1}{\rho r}\frac{\partial p}{\partial \theta} + f_\theta + \frac{\mu}{\rho}\left(\nabla^2 V_\theta - \frac{V\theta}{r^2} + \frac{2}{r^2}\frac{\partial V_r}{\partial \theta}\right) \tag{11}$$

$$\frac{\partial V_z}{\partial t} + (\boldsymbol{V} \cdot \nabla)V_z = -\frac{1}{\rho}\frac{\partial p}{\partial z} + f_z + \frac{\mu}{\rho}\nabla^2 V_z \tag{12}$$

[2] The source for Eqs. (3) through (11) is Issacs et al. (2012).

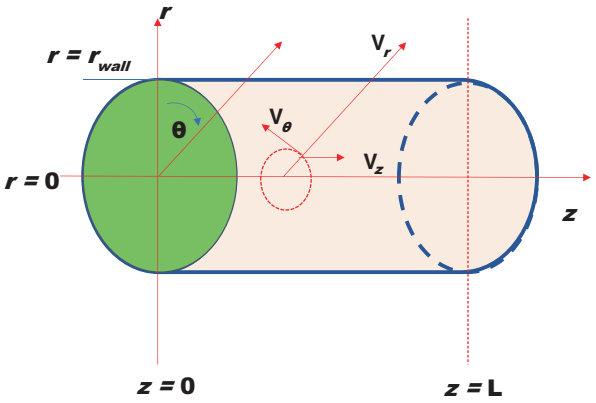


Fig. 6 Coordinate system for an ideal cylindrical airway, depicting velocity component at an arbitrary point. (Sources of information: Vallero 2014; Issacs et al. 2012)

Where,

$$\boldsymbol{V} \cdot \nabla = V_r \frac{\partial}{\partial r} + \frac{1}{r} V_\theta \frac{\partial}{\partial \theta} + V_z \frac{\partial}{\partial z} \tag{13}$$

The first terms (i.e. time derivatives) in these three equations can be ignored under steady state conditions. The Laplacian operator can be defined in cylindrical airways as:

$$\nabla^2 = \frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial}{\partial r} \right) + \frac{1}{r^2} \frac{\partial^2}{\partial \theta^2} + \frac{\partial^2}{\partial z^2} \tag{14}$$

Airway velocities are complicated by numerous factors including lung and other tissue morphologies, and the airway generations, i.e. levels of branching level through which the air is flowing. This is one possible area where the fluid dynamics of engineered nanoparticles and ultrafines may differ from most other aerosols, i.e. their very small size may lead them to resemble a gas-phase substance more than an aerosol, so they may either penetrate more deeply, which would worsen the exposure, or conversely they may be more efficiently exhaled, having never been absorbed. Other factors would likely determine which of these would hold. For example, if they behave as surfactants, they may readily dissolve into lung tissue. If they are relatively soluble in tissue or blood, they may also be absorbed and distributed efficiently, possibly leading to more toxic effects. However, if the physicochemical properties allow them to be readily hydrolized, they may be more efficiently undergo phase 1 metabollism and elimination.

Equations can be tailored to these conditions or idealized velocity profiles can be assumed for the cascade of generations. These include parabolic flow (laminar fully developed), plug flow (laminar undeveloped) and turbulent flow (Issacs et al. 2012).

For example, the upper tracheobronchial airways may be assumed to be turbulent, but in the pulmonary region, plug and parabolic profiles may be assumed.

The right and left lung are connected via their primary bronchi to the trachea and upper airway of the nose and mouth (see Fig. 4). From there, the bronchi, i.e. airways, subdivide into a branching network of many levels. Each level, called a generation, is designated with an integer. The trachea are generation $n = 0$), the primary bronchi are generation $n = 1$, and so forth. Thus, theoretically there are $2n$ airway tubes at generation n. In the conducting zone (i.e. generations $0 \leq n \leq 16$), air flow is restricted to entry and exit in the airway (Grotberg 2011). That is, air is moving, but there is no air-blood gas exchange of O_2 and CO_2.

Air exchange occurs in generations $n > 16$, known as the respiratory zone. Generations $17 \leq n \leq 19$ are the locations of the airway walls' air sacs (alveoli), which range from 75 to 300 μm in diameter (Grotberg 2011). Alveoli are thin-walled and, owing to the rich capillary blood supply in them, are designed for gas exchange. The respiratory bronchioles are the vessels by which air passes to alveoili. The walls of the tubes or ducts in generations $20 \leq n \leq 22$, consist entirely of alveoli. At generation $n = 23$, terminal alveolar sacs are made up clusters of alveoli (Issacs et al. 2012).

Two principal factors are relevant to gas exchange are the airway volume (V_{aw}) and airway surface area (A_{aw}), which are proportional to the size of the person. Air exchange increases in proportion to A_{aw}. The V_{aw} (mL) for children is proportional to height and is approximated as (Kerr 1976):

$$V_{aw} = 1.018 \times \text{Height}\left(\text{cm}\right) - 76.2 \tag{15}$$

V_{aw} (ml) can be estimated for adults by adding the ideal body weight (pounds) plus age in years (Bouhuys 1964). For example, a 40-year-old adult whose ideal body weight is 160 pounds has an estimated V_{aw} of 200 mL (George and Hlastala 2011).

The average human lung has from 300 to 500 million of these air sacs. In an average adult lung, the total alveolar surface area is 70 m^2. This large A_{aw} allows for efficient gas exchange to supply O_2 for normal respiration but also large increases in gas exchange needed when a person is stressed (e.g. during exercise, injury or illness). The Reynolds number varies by generation (very high in the trachea, but low in the alveoli) (Grotberg 2011). Airways have liquid lining, with two layers in the first generations (up to about $n = 15$). A watery, serous layer is next to the airway wall; behaving as a Newtonian fluid. This layer has cilia that pulsate toward the mouth. Atop the serous layer is a mucus layer that possesses several non-Newtonian fluid properties, e.g. viscoelasticity, shear thinning, and a yield stress.

Alveolar cells produce surfactants that orient at the air-liquid interface and reduce the surface tension significantly. Air pollutants can adversely affect the surfactant chemistry, which can make the lungs overly rigid, thus hindering inflation (Grotberg 2011). A pulmonary surfactant is a surface-active lipoprotein complex (phospholipoprotein) produced by type II pneumocytes, which are also known as

alveolar type II cells. These pneumocytes are granular and comprise 60% of the alveolar lining cells. Their morphology allows them to cover smaller surface areas than type I pneumocytes. Type 1 cells are highly attenuated, very thin (25 nm) cells that line the alveolar surfaces and cover 97% of the alveolar surface. Surfactant molecules have both a hydrophilic head and a lipophilic tail. Surfactants adsorb to the air-water interface of the alveoli with the hydrophilic head collects in the water, while the hydrophobic tail is directed towards the air. The principal lipid component of dipalmitoylphosphatidylcholine, a surfactant that decreases surface tension. The actual surface tension decreases depends the surfactant's concentration on the interface. This concentration's saturation limit depends on temperature and the presence of other compounds in the interface. Surface area of the lung varies during compliance (i.e. lung and thorax expansion and contraction) during ventilation. Thus, the surfactant's interface concentration is seldom at the level of saturation. During lung expansion (inspiration), the surface increases, opening space for new surfactant molecules to join the interface mixture. During expiration, lung surface area decreases, compressing the surfactant and increasing the density of surfactant molecules, thus further decreasing the surface tension. Therefore, surface tension varies with air volume in the lungs, which protects the lungs from atelectasis at low air volume and form tissue damage at high air volume (Schurch et al. 1992).

Another difference between engineered nanomaterial and larger scale PM is the role of diffusion. Indeed, Fickian diffusion is important only for nanoscale particles ($\leq$0.1 μm diameter) because the Brownian motion allows them to move in a "random walk" away from the air stream. Thus, even though air pollution toxicology usually ignores molecular diffusion as an important transport mechanism, this assumption does not hold for ultrafine PM and engineered nanomaterials.

Other transport mechansims are also important following aggregaton or particle growth (Cigánková et al. 2021). Indeed, certain sections of the respiratory system behave as a filter (see Fig. 7). Interception begins when the aggregates reach diameters between 0.1 and 1 μm. As such, impaction is one mechanism for control of released nanomaterials, because the aggregate does not leave the air stream but comes into contact with an obstacle (e.g. respiratory cilia). Inertial impaction collects aerosols that are sufficiently large to leave the air stream.

by inertia (diameters $\geq$ 1 μm). Electrostatics consist of electrical interactions between the atoms in tissure and those in the particle at the point of contact (Van der Waal's forces), as well as electrostatic attraction (charge differences between particle and tissure). Other important factors affecting filtration efficiencies include the thickness and pore diameter or the filter, the uniformity of particle diameters and pore sizes, the solid volume fraction, the rate of particle loading (e.g. affecting particle "bounce"), the particle phase (liquid or solid), capillarity and surface tension, and characteristics of air or other carrier gases, such as velocity, temperature, pressure, and viscosity. The nanoparticle clearance is affected by the pulmonary surfactant, i.e.the mixture of lipids and proteins secreted by the epithelial type II cells into the alveolar space (Schurch et al. 1992; Veldhuizen and Haagsman 2000). The surfactant reduces the surface tension at the liquid-air interface in the lung by forming a surface film. Thus, the nanomaterial is sorbed and becomes dissolved or suspended

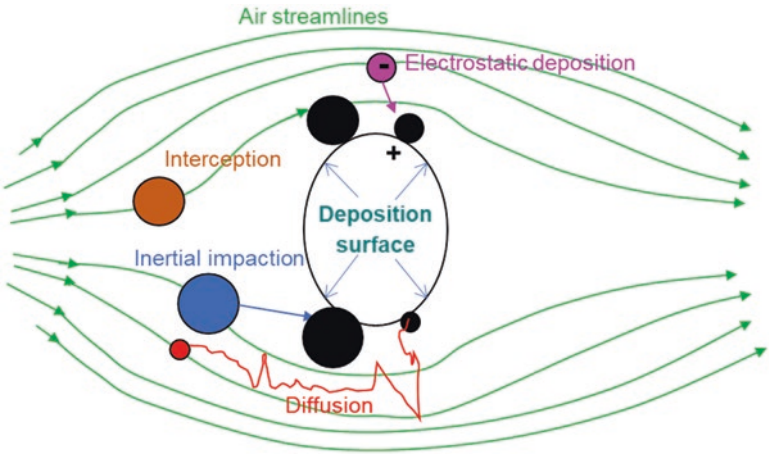


Fig. 7 Mechanical processes leading to the deposition of particulate matter. Diffusion can be an important filtration mechanism for nanoparticles, but the other factors increase in importance with aggregation. (Sources of information: Vallero 2013, 2014; Rubow et al. 2004)

in this film. During breathing including, amongst others, selective adsorption of dipalmitoylphosphatidylcholine (DPPC)-enriched domains, specific release of non-DPPC lipids, lipid reservoir formation and modulation of monolayer packing (Veldhuizen and Haagsman 2000). Each process likely needs a combination of both the lipid and protein components of the surfactant.

Much of the toxicology of particles has been associated with inhalation exposures. Inhalation exposure (E) can be expressed as (Vallero 2014; Derelanko 2014):

$$E = \frac{(C)\cdot(PC)\cdot(IR)\cdot(RF)\cdot(EL)\cdot(AF)\cdot(ED)\cdot(10^{-6})}{(BW)\cdot(TL)} \tag{16}$$

Where,

C = concentration of the contaminant on the aerosol/particle (mg kg^{-1})
PC = particle concentration in air (gm m^{-3})
IR = inhalation rate (m^3 h^{-1})
RF = respirable fraction of total particulates (dimensionless, usually determined by aerodynamic diameters, e.g. 2.5 μm)
EL = exposure length (h d^{-1})
ED = duration of exposure (d)
AF = absorption factor (dimensionless)
BW = body weight (kg)
TL = typical lifetime (d).
10^{-6} is a conversion factor (kg to mg)

The human body and other biological systems have a capacity for the uptake of myriad types of substances and utilize them to support some bodily function or eliminate them. In work or exercise scenarios, for example, the exposure to NPs is greatly increased because of the elevated IR and PC values.

The quality and amount of data from which to base nanomaterial exposures varies. As analytical capabilities have improved, increasingly lower concentrations of chemicals have been observed in various parts of the body. Some of these chemicals enter the body by inhalation, whereas the dominant pathway for others could be in drinking water, food, skin contact. Equations for each of these pathways are analogous to Eq. (16). However, observing nanoparticles in various body tissues is very uncertain since the particles often will have changed after absorption, e.g. undergoing aggregation and metabolism (Darquenne 2012; Fröhlich and Salar-Behzadi 2014; Isaacs et al. 2012; MacCuspie et al. 2011; Rogers et al. 2012). What may have been inhaled at the nanoscale, may change to larger particles, may react and become organometallic compounds, or may remain nanoparticles in suspension (Li et al. 2011).

Toxicokinetics

Endogenously, varying amounts of the parent substance (e.g. zero-valent metal), any salts and ions formed, and other chemical species (e.g. organometallic compounds) are absorbed and distributed within the body. For inorganic metallic NPs, the principal difference between the partitioning of that nanomaterials versus other forms of the metal is the distribution among zero-valence, ions and metallic compounds. However, within certain organic solvents e.g. metallic NP additives in diesel fuel, the distributions will be among metallic NPs, organometallic compounds and larger suspended particles prior to combustion (Vallero 2012). Given the relatively large specific surface area compared to that of $PM_{2.5}$, a nanoparticle or an ultrafine has exponentially more potentially active sorption and solution sites. The low mass of the NP means that it can remain suspended for a very long time. Such nano-suspensions in surface waters allows the even heavy metal-laden NPs to remain in the water column, rather than settle onto the surface; increasing the likelihood that the metal will be exposed to free oxygen than to the more reduced and anoxic conditions of the sediment. In the air, these features mean that the NP will be more likely to remain airborne for longer time periods and to undergo atmospheric transformation.

These differences in mass and volume from bulk materials can also translate into endogenous differences, meaning that absorption, distribution, metabolism, excretion, and toxicity could also be different for NPs compared to bulk materials and larger PM. Based on pharmaceutical research, some of the factors that lead to these differences, in addition to size, are surface charge and surface chemistry (Li and Huang 2008; Elci et al. 2016). The fraction of the metal species or its transformation products that accumulates in lipids and other tissue substrates could be higher, and

the amount excreted decreased, so that the difference results in bioaccumulation and increased body burden (see Fig. 8). Indeed, this is an example of how the drug delivery and pollutant toxicity research are mutually supportive, albeit for opposite ends. That is, pharmaceutical research seeks to increase adsorption and minimize elimination of the active ingredient, whereas toxicokinetic research related to detoxification, e.g. prevention of a xenobiotic toxin from reaching vulnerable tissue, aims to decrease absorption and to maximize elimination.

The metal NP, cations, and its metabolites thereafter induce toxicity in various ways. For toxicity (e.g. metal-induced neuropathologies) to occur, a metal must reach a target (e.g. a neuron) at a concentration sufficient to alter mechanistically

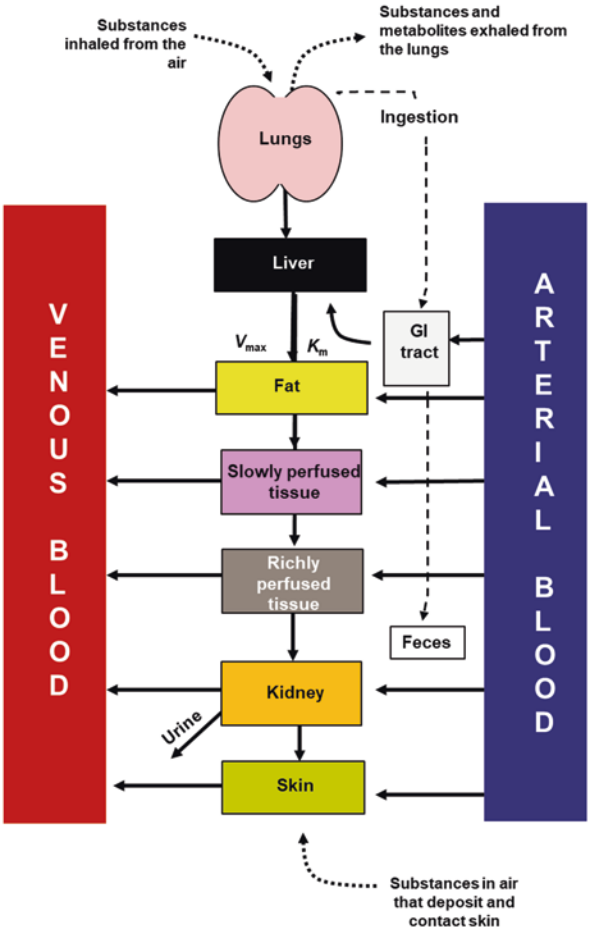


Fig. 8 Toxicokinetics for a hypothetical nanomaterial that has been inhaled, ingested or contacted dermally. Note: V_{max} is the maximum velocity at which the enzyme catalyzes a reaction, and K_m is the substrate concentration that is required for the reaction to occur at one-half of $V_{max.}$ (Sources of information: Vallero 2014; Agency for Toxic Substances and Disease Registry 2002)

the normal functioning of the tissue. Metal toxicity can involve types of membrane receptor-ligand disruptions. However, it may also damage intracellular receptors and ion channels. Metals tend to react with nucleophilic macromolecules, e.g. proteins, amino acids and nucleic acids. A nucleophile donates an electron pair to an electrophile, an electron pair acceptor, to form a chemical bond. An NP, for example, may react with sulfur (S) in thiols, cysteinyl protein residues and glutathione and S in thiols and thiolates. However, some metals, e.g. lithium, calcium and barium, preferentially react with harder nucleophiles, e.g. the oxygen in purines. Lead tends to exhibit universal reactivity with all nucleophiles (Shanker 2008). The most damaging adverse outcome pathway for metallic NPs and metal oxides is ionic due to reactive oxygen species (ROS) and cellular membrane damage (Beer et al. 2012; Poynton et al. 2010, 2012).

Again, these effects have been observed in metals and metalloids in various forms, with nanomaterials playing a role of either degrading or improving environmental conditions. How metal NPs differ is a subject of current research. In addition, metals in various forms and sizes are influenced by the presence of NPs. For example, Pb mobility and bioavailability can be adjusted by inserting Fe NPs (e.g. $Fe_3(PO_4)_2{\cdot}8H_2O$) into Pb-contaminated soil, i.e. converting highly aqueous soluble and exchangeable forms to less soluble and less exchange forms (Liu and Zhao 2007). Such findings can greatly improve environmental remediation efforts.

Exposure Models

Risk management depends on models to estimate exposures. Such models range from "screening-level" to "high-tiered." Screening models generally over-predict exposures because they are based on conservative default values and assumptions. They provide a first approximation that screens out exposures not likely to be of concern (Judson et al. 2010; U.S. Environmental Protection Agency 2017b; Guy et al. 2008; Zhang et al. 2014; Chemical Computing Group 2013; Hilton et al. 2010). Conversely, higher-tiered models typically include algorithms that provide specific site characteristics, time-activity patterns, and are based on relatively realistic values and assumptions. Such models require data of higher resolution and quality than the screening models and, in return, provide more refined exposure estimates (U.S. Environmental Protection Agency 2017b).

Environmental stressors can be modeled in a unidirectional and one-dimension fashion. A conceptual framework can link exposure to environmental outcomes across levels of biological organization (Fig. 9). Thus, environmental exposure assessment considers coupled networks that span multiple levels of biological organization that can describe the interrelationships within the biological system. Mechanisms can be derived by characterizing and perturbing these networks, e.g. behavioral and environmental factors (Hubal et al. 2010). This can apply to a food chain or food web model (Fig. 10) or a kinetic model Fig. 11) or numerous other modeling platforms.

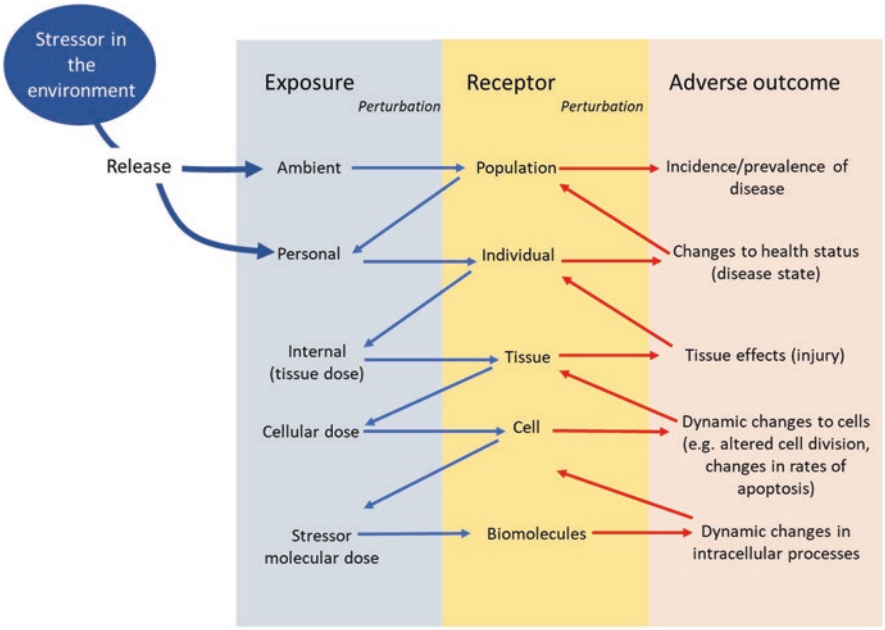


Fig. 9 Systems cascade of exposure-response processes. In this instance, scale and levels of biological organization are used to integrate exposure information with biological outcomes. The stressor (chemical or biological agent) moves both within and among levels of biological organization, reaching various receptors, thereby influencing and inducing outcomes. The outcome can be explained by physical, chemical and biological processes (e.g. toxicogenomic mode-of-action information). (Source of information: Hubal et al. 2010)

Exposure Estimation

Exposure results from sequential and parallel processes in the environment, from release to environmental partitioning to movement through pathways to uptake and fate in the organism (see Fig. 12). The substances often change to other chemical species as a result of the body's metabolic and detoxification processes. Certainly, genetic modifications can affect such processes. New substances, known as degradation products or metabolites, are produced as cells use the parent compounds as food and energy sources. These metabolic processes, such as hydrolysis and oxidation, are the mechanisms by which chemicals are broken down.

The exposure pathway also includes the ways that humans and other organisms can come into contact with the hazard The pathway has five parts:

1. The source of contamination (e.g. fugitive dust or leachate from a landfill)
2. An environmental medium and transport mechanism (e.g. soil with water moving through it)
3. A point of exposure (such as a well used for drinking water)

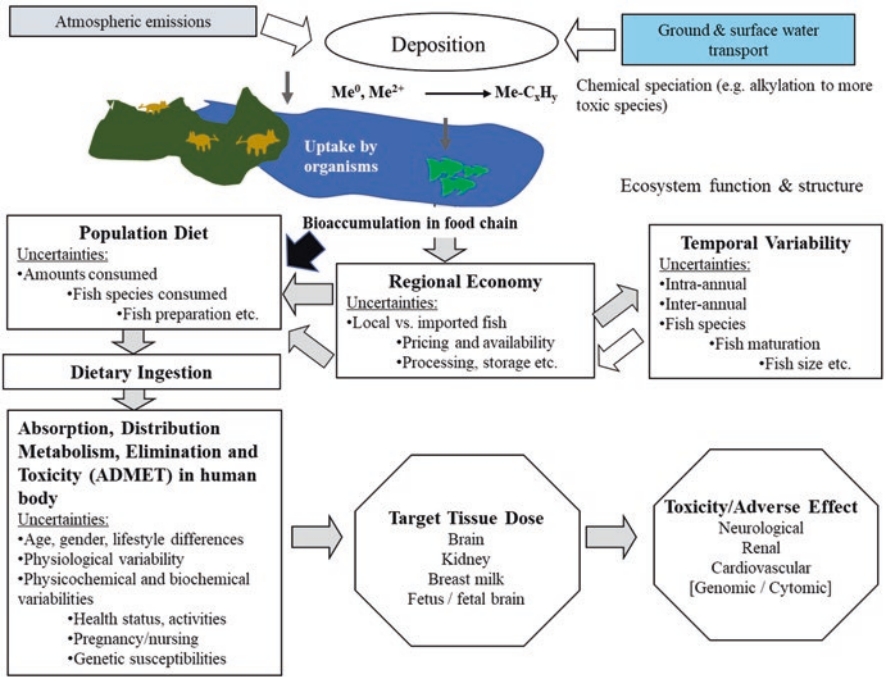


Fig. 10 Biochemodynamic pathways for a substance (in this case a single chemical compound). The fate is mammalian tissue. Various modeling tools are available to characterize the movement, transformation, uptake and fate of the compound. Similar biochemodynamic paradigms can be constructed for multiple chemicals (e.g. mixtures) and microorganisms. (Source of information: Vallero 2010b)

4. A route of exposure (e.g. inhalation, dietary ingestion, nondietary ingestion, dermal contact, and nasal)
5. A receptor population (those who are actually exposed or who are where there is a potential for exposure)

If all five parts are present, the exposure pathway is known as a completed exposure pathway. In addition, the exposure may be short-term, intermediate, or long-term. Short-term contact is known as an acute exposure, i.e. occurring as a single event or for only a short period of time (up to 14 days). An intermediate exposure is one that lasts from 14 days to less than 1 year. Long-term or chronic exposures are greater than 1 year in duration.

Determining the exposure for a neighborhood can be complicated. For example, even if all of the contaminants of concern can be identified, along with their possible source (no small task), we may have little idea of the extent to which the receptor population has come into contact with these contaminants (steps 2 through 4). Thus, assessing exposure involves not only the physical sciences, but the social sciences, e.g. psychology and behavioral sciences. People's activities greatly affect the amount and type of exposures. That is why exposure scientists use a number of

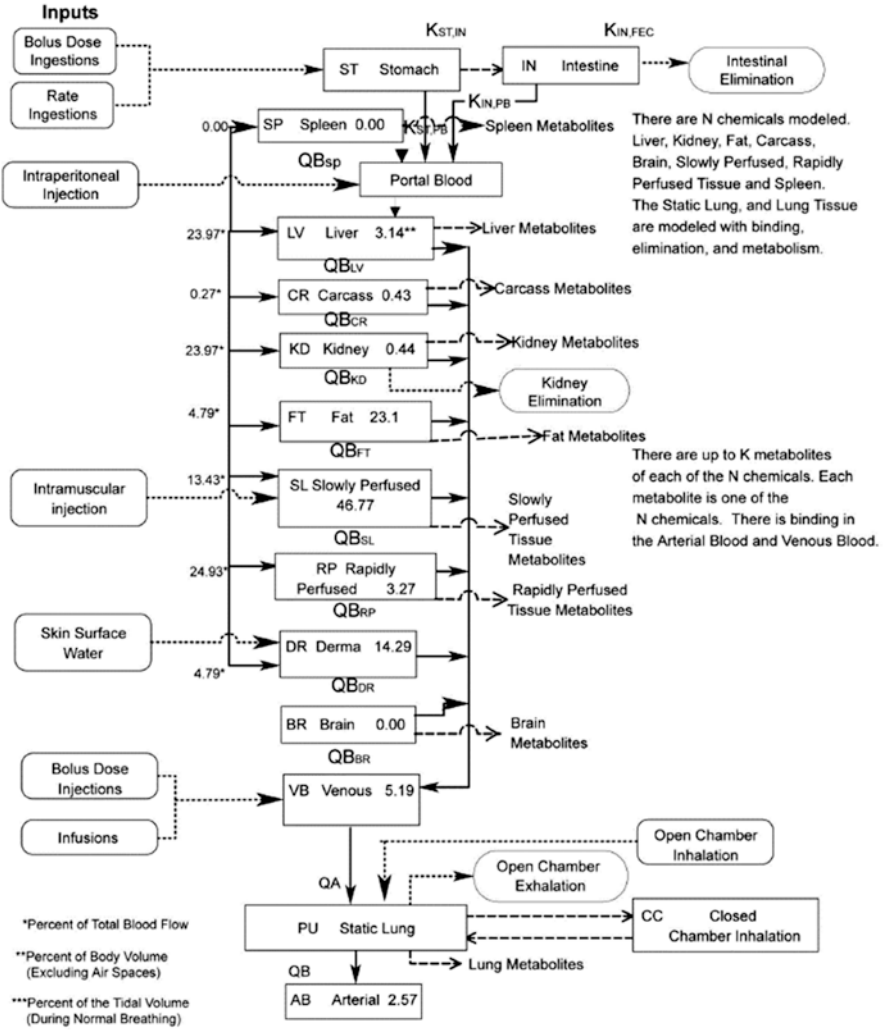


Fig. 11 Toxicokinetic model used to estimate dose as part of an environmental exposure. This diagram represents the static lung, with each of the compartments (brain, carcass, fat, kidney, liver, lung tissue, rapidly and slowly perfused tissues, spleen, and the static lung) having two forms of elimination, an equilibrium binding process, and numerous metabolites. Notes: K refers to kinetic rate; Q to mass flow; and Q_B to blood flow. A breathing lung model would consist of alveoli, lower dead space, lung tissue, pulmonary capillaries, and upper dead space compartments. Gastrointestinal (GI) models allow for multiple circulating compounds with multiple metabolites entering and leaving each compartment, i.e. the GI model consists of the wall and lumen for the stomach, duodenum, lower small intestine, and colon, with lymph pool and portal blood compartments included. Bile flow is treated as an output from the liver to the duodenum lumen. All uptaken substances are treated as circulating. Nonspecific ligand binding, e.g. plasma protein binding, is represented in arterial blood, pulmonary capillaries, portal blood, and venous blood. (Source: Dary et al. 2007. Adapted from: Blancato et al. 2006)

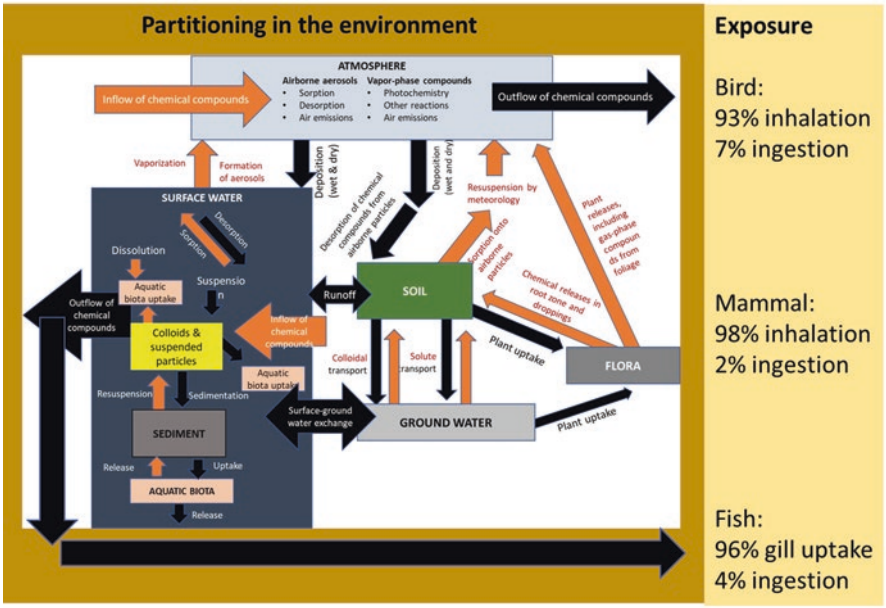


Fig. 12 Processes leading to organismal uptake and fate of chemical and biological agents after release into the environment. In this instance, the predominant sources are air emissions, and predominant pathway of exposure is inhalation. However, due to deposition to surface waters and the agent's affinity for sediment, the ingestion pathways are also important. Dermal pathways, in this case, do not constitute a large fraction of potential exposure. (Source: McKone et al. 2006)

techniques to establish activity patterns, such as asking potentially exposed individuals to keep diaries, videotaping, and using telemetry to monitor vital information, e.g. heart and ventilation rates.

General ambient measurements, such as air pollution monitoring equipment located throughout cities, are often not good indicators of actual population exposures. For example, metals and their compounds comprise the greatest mass of toxic substances *released* into the U.S. environment. This is largely due to the large volume and surface areas involved in metal extraction and refining operations. However, this does not necessarily mean that more people will be exposed at higher concentrations or more frequently to these compounds than to others. A substance that is released or even that if it resides in the ambient environment is not tantamount to its coming in contact with a *receptor*. Conversely, even a small amount of a substance under the right circumstances can lead to very high levels of exposure (e.g. handling raw materials and residues at a waste site).

The simplest quantitative expression of exposure is:

$$E = D / t \tag{17}$$

where E is the human exposure during the time period t (units of concentration.

(mg kg^{-1} d^{-1}); D is the mass of pollutant per body mass (mg kg^{-1}); and t is time (day).

D, the chemical concentration of a pollutant, is usually measured near the interface of the person and the environment, during a specified time-period. This measurement is sometimes referred to as the potential dose (i.e., the chemical has not yet crossed the boundary into the body, but is present where it may enter the person, such as on the skin, at the mouth, or at the nose).

Expressed quantitatively, exposure is a function of the concentration of the agent and time. It is an expression of the magnitude and duration of the contact. That is, exposure to a contaminant is the concentration of that contact in a medium integrated over the time of contact:

$$E = \int_{t=t_1}^{t=t_2} C(t)\,dt \tag{18}$$

where, E is the exposure during the time period from t_1 to t_2, and $C(t)$ is the concentration at the interface between the organism and the environment, at time t.

The concentration at the interface is the potential dose (i.e., the agent has not yet crossed the boundary into the body, but is present where it may enter the receptor). Since the amount of a chemical agent that penetrates from the ambient atmosphere into a control volume affects the concentration term of the exposure equation, a complete mass balance of the contaminant must be understood and accounted for; otherwise exposure estimates will be incorrect. Recall that the mass balance consists of all inputs and outputs, as well as chemical changes to the contaminant:

$$\begin{aligned}&\text{Accumulation or loss of contaminant A} = \text{Mass of A transported in}\\&-\text{Mass of A transported out} \pm \text{Reactions}\end{aligned} \tag{19}$$

The reactions may be either those that generate substance A (i.e. *sources*), or those that destroy substance A (i.e. *sinks*). Thus, the amount of mass transported in is the inflow to the system that includes pollutant discharges, transfer from other control volumes and other media (for example, if the control volume is soil, the water and air may contribute mass of chemical A), and formation of chemical A by abiotic chemistry and biological transformation. Conversely, the outflow is the mass transported out of the control volume, which includes uptake, by biota, transfer to other compartments (e.g. volatilization to the atmosphere) and abiotic and biological degradation of chemical A. This means the rate of change of mass in a control volume is equal to the rate of chemical A transported in less the rate of chemical A transported out, plus the rate of production from sources, and minus the rate of elimination by sinks. Stated as a differential equation, the rate of change contaminant A is:

$$\frac{d[A]}{dt} = -v \cdot \frac{d[A]}{dx} + \frac{d}{dx}\left(\Gamma \cdot \frac{d[A]}{dx}\right) + r \tag{20}$$

where v is the fluid velocity; Γ is a rate constant specific to the environmental medium; $\frac{d[A]}{dx}$ is the concentration gradient of chemical A; and r refers to the internal sinks and sources within the control volume.

Reactive compounds can be particularly difficult to measure. For example, many volatile organic compounds in the air can be measured by first collecting in stainless steel canisters and analyzed by chromatography in the lab. However, some of these compounds, like the carbonyls (notably aldehydes like formaldehyde and acetaldehyde) are prone to react inside the canister, meaning that by the time the sample is analyzed a portion of the carbonyls are degraded (under-reported). Therefore, other methods, such as trapping the compounds with dinitrophenyl hydrazine (DNPH) treated silica gel tubes that are frozen until being extracted for chromatographic analysis. The purpose of the measurement is to see what is in the air, water, soil, sediment, or biota at the time of sampling, so any reactions before the analysis give measurement error.

The general exposure Eq. (7) is rewritten to address each route of exposure, accounting for chemical concentration and the activities that affect the time of contact. The exposure calculated from these equations is actually the chemical intake (I) in units of concentration (mass per volume or mass per mass) per time, such as mg kg^{-1} d^{-1}:

$$I = \frac{C \cdot CR \cdot EF \cdot ED \cdot AF}{BW \cdot AT} \tag{21}$$

Where C is the chemical concentration of contaminant (mass per volume); CR is the contact rate (mass per time); EF is the exposure frequency (number of events, dimensionless);and ED is the exposure duration (time).

These factors are further specified for each route of exposure, such as the lifetime average daily dose (LADD) as shown in Table 4. The LADD is obviously based on a chronic, long-term exposure.

Acute and subchronic exposures require different equations, since the exposure duration (ED) is much shorter. For example, instead of LADD, acute exposures to non-carcinogens may use maximum daily dose (MDD) to calculate exposure (see Discussion Box). However, even these exposures follow the general model given in Eq. (15).

Hypothetical Example of an Exposure Calculation*In the process of synthesizing pesticides over an 18-year period, a polymer manufacturer has contaminated the soil on its property with a nanoparticle. The plant closed two years ago but vinyl chloride vapors continue to reach the neighborhood surrounding the plant at an average concentration of 1 mg* m^{-3}*. Assume that people are breathing at a ventilation rate of 0.5* m^3 h^{-1} *(about the average of adult males and females over 18 years of age (***Moya et al. 2011***). The legal settlement allows neighboring residents to evacuate and sell their homes to the company. However, they may also stay. The neighbors have asked for advice on whether to stay or leave, since they have already been exposed for*

20 years. **is highly volatile, so its phase distribution will be mainly in the gas phase rather than the phase. Although some of the vinyl chloride may be sorbed to particles, we will use only vapor phase equation, since the particle phase is likely to be relatively small. Also, we will assume that outdoor concentrations are the exposure . This is unlikely, however, since people spend very little time outdoors compared to indoors, so this may provide an additional factor of safety. To determine how much vinyl chloride penetrates living quarters, indoor air studies would have to be conducted. For a scientist to compare exposures, indoor air measurements should be taken.Find the appropriate equation in Table 4 and insert values for each variable. are published by the EPA and the Oak Ridge National Laboratory's Risk Assessment Information System (http://risk.lsd.ornl.gov/cgi-bin/tox/TOX_select?select=nrad). Vinyl chloride is well absorbed, so for a worst case we can that AF = 1. We will also assume that the person stays in the neighborhood is exposed at the average concentration 24 hours a day (EL = 24), and that a person lives the remainder of entire typical lifetime exposed at the measured concentration.Although the ambient concentrations of may have been higher when the plant was operating, the only measurements we have are those taken recently. Thus, this is an area of uncertainty that must be discussed with the clients. The common default value for a lifetime is 70 years, so we can assume the longest exposure would be is 70 years (25,550 days). Table 5 gives some of the commonly used default values in exposure assessments. If the person is now 20 years of age and has already been exposed for that time, and lives the 50 years exposed at 1 mg m^{-3}:**

$$\begin{aligned}\text{LADD} &= \frac{(C)\cdot(IR)\cdot(EL)\cdot(AF)\cdot(ED)}{(BW)\cdot(TL)} \\ &= \frac{(1)\cdot(0.5)\cdot(24)\cdot(1)\cdot(25550)}{(70)\cdot(25550)} \\ &= 0.2\,\text{mg}\,\text{kg}^{-1}\,\text{day}^{-1}\end{aligned}$$

If the 20 year old leaves today, the exposure duration would be for the 20 years that the person lived in the neighborhood. Thus, only the ED term would change, i.e. from 25,550 days to 7300 days (i.e. 20 years).Thus, the falls to 2/7 of its value:LADD = 0.05 mg kg^{-1} day^{-1}Note that this is a straightforward, chemical exposure estimate in the gas phase. Often, a chemical will exist as a vapor, an aerosol, or sorbed to an aerosol. In this case, the inhalation exposure would have to be calculated for the gas and the , i.e. the

concentration of PM and the concentration of the chemical in the PM (the first two equations in Table 4). Furthermore, if this were an exposure involving an emerging technology, it would be much more complex and uncertain, since the routes and pathway information may be more difficult to ascertain, e.g. the exposure and toxicity of nanomaterials will include factors other than their inherent chemical composition. The risk assessment may even have greater, since it is likely that at least some of the particle size, shape and coatings may largely affect potential toxicity and hazard, so even if the exposure probabilty is reliable, the risk assessment will be weakened if only based on published chemical toxicity and exposure data.Once the hazard and are done, risks can be characterized risk quantitatively. There are two general ways that such risk characterizations are used in environmental problem solving, i.e. direct risk assessments and risk-based cleanup standards.

Conclusions

Risk management decisions must be underpinned by scientifically credible and reliable assessments of both the hazards and the likelihood and extent of exposure to those hazards. Thus, reliable exposure estimates are required for decisions involving synthetic biology and emerging technologies.

This chapter introduced exposure assessment approaches, identifying where larger scale mechanisms may apply and those that differ for nanomaterials. These also apply to ways to reduce exposures, incluing particle collection technologis. There is much uncertainty as to the efficiency of these technologies and conventional methods may fail due not only due to the small size and mass of nanoparticles, but also to their chemical makeup and other physicochemical properties.

Acknowledgments and Disclaimer Kim Rogers and Caroline Stevens of EPA's National Exposure Research Laboratory provided substantive reviews and technical recommendations that enhanced this chapter. This document has been reviewed in accordance with the U.S. EPA policy and approved for publication. Mention of trade names commercial products does not constitute endorsement nor recommendation for use. The views expressed in this journal article are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

Table 4 Equations for calculating lifetime average daily dose (LADD) for various routes of exposure

Route of Exposure	Equation LADD (in mg kg^{-1} d^{-1}) =	Definitions
Inhaling aerosols (particulate matter)	$\frac{(C)\cdot(PC)\cdot(IR)\cdot(RF)\cdot(EL)\cdot(AF)\cdot(ED)\cdot(10^{-6})}{(BW)\cdot(TL)}$	C = concentration of the contaminant on the aerosol/particle (mg kg^{-1}) PC = particle concentration in air (gm m^{-3}) IR = inhalation rate (m^{-3} h^{-1}) RF = respirable fraction of total particulates (dimensionless, usually determined by aerodynamic diameters, e.g. 2.5 μm) EL = exposure length (h d^{-1}) ED = duration of exposure (d) AF = absorption factor (dimensionless) BW = body weight (kg) TL = typical lifetime (d). 10^{-6} is a conversion factor (kg to mg)
Inhaling vapor phase contaminants	$\frac{(C)\cdot(IR)\cdot(EL)\cdot(AF)\cdot(ED)}{(BW)\cdot(TL)}$	C = concentration of the contaminant in the gas phase (mg m^{-3}) Other variables the same as above.
Drinking water	$\frac{(C)\cdot(CR)\cdot(ED)\cdot(AF)}{(BW)\cdot(TL)}$	C = concentration of the contaminant in the drinking water (mg L^{-1}) CR = rate of water consumption (L d^{-1}) ED = duration of exposure (d) AF = portion (fraction) of the ingested contaminant that is physiologically absorbed (dimensionless) Other variables are the same as above.
Contact with soil-borne contaminants	$\frac{(C)\cdot(SA)\cdot(BF)\cdot(FC)\cdot(SDF)\cdot(ED)\cdot(10^{-6})}{(BW)\cdot(TL)}$	C = concentration of the contaminant in the soil (mg kg^{-1}) SA = skin surface area exposed (cm^{-2}) BF = bioavailability (percent of contaminant absorbed per day) FC = fraction of total soil from contaminated source (dimensionless) SDF = soil deposition, the mass of soil deposited per unit area of skin surface (mg cm^{-1} d^{-1}) Other variables are the same as above.

Source: Derelanko 1999

Table 5 Commonly used human exposure factors

Exposure factor	Adult male	Adult female	Child (3–12 years of age)
Body weight (kg)	70	60	15–40
Total fluids ingested (L d^{-1})	2	1.4	1.0
Surface area of skin, without clothing (m^2)	1.8	1.6	0.9
Surface area of skin, wearing clothes (m^2)	0.1–0.3	0.1–0.3	0.05–0.15
Respiration/ventilation rate (L min^{-1}) – Resting	7.5	6.0	5.0
Respiration/ventilation rate (L min^{-1}) – Light activity	20	19	13
Volume of air breathed (m^3 d^{-1})	23	21	15
Typical lifetime (years)	70	70	NA
National upper-bound time (90th percentile) at one residence (years)	30	30	NA
National median time (50th percentile) at one residence (years)	9	9	NA

Sources of data: U.S. Environmental Protection Agency, 2003, *Exposure Factor Handbook* (Moya et al. 2011); and Agency for Toxic Substances and Disease Registry, 2003, ATSDR Public Health Assessment Guidance Manual (Agency for Toxic Substances and Disease Registry 2005)

References

Aberg B, Ekman L, Falk R, Greitz U, Persson G, Snihs JO. Metabolism of methyl mercury (203Hg) compounds in man: excretion and distribution. Arch Environ Health Int J. 1969;19(4):478–84.

Agency for Toxic Substances and Disease Registry. Toxicological Profile for DDT, DDE, and DDD. In: Agency for toxic substances and disease registry PHS, U.S. Department of Health and Human Services, editor. Atlanta; 2002.

Agency for Toxic Substances and Disease Registry. Public health assessment guidance manual. Centers for Disease Control and Prevention. 2005.

Anastas PT, Lankey RL. Life cycle assessment and green chemistry: the yin and yang of industrial ecology. Green Chem. 2000;2(6):289–95.

Arnaldi S, Muratorio A. Nanotechnology, uncertainty and regulation: A guest editorial. Nanoethics 2013;7:173–175. https://doi.org/10.1007/s11569-013-0185-3.

Barber MC, Isaacs KK, Tebes-Stevens C. Developing and applying metamodels of high resolution process-based simulations for high throughput exposure assessment of organic chemicals in riverine ecosystems. Sci Total Environ. 2017;605:471–81.

Beaudrie CE, Kandlikar M, Gregory R, Long G, Wilson T. Nanomaterial risk screening: a structured approach to aid decision making under uncertainty. Environ Syst Decis. 2015;35(1):88–109.

Beer C, Foldbjerg R, Hayashi Y, Sutherland DS, Autrup H. Toxicity of silver nanoparticles—nanoparticle or silver ion? Toxicol Lett. 2012;208(3):286–92.

Benson B, Mills A, Wood B, editors. A review of the reference dose and reference concentration processes. Risk Assessment Forum EPA/630/P-02 F; 2002.

Bergin IL, Witzmann FA. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. Int J Biomed Nanosci Nanotechnol. 2013;3(1–2):163–210.

Birnbaum LS, Jung P. From endocrine disruptors to nanomaterials: advancing our understanding of environmental health to protect public health. Health Aff. 2011;30(5):814–22.

Blancato JN, Power FW, Brown RN, Dary CC. Exposure related dose estimating model (ERDEM): a physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) model for assessing human exposure and risk. Report no. EPA/600/R-06/061. Las Vegas: U.S. Environmental Protection Agency; 2006.

Boonyaroj V, Chiemchaisri C, Chiemchaisri W, Yamamoto K. Enhanced biodegradation of phenolic compounds in landfill leachate by enriched nitrifying membrane bioreactor sludge. J Hazard Mater. 2017;323:311–8.

Bouhuys A. Respiratory dead space. In: Handbook of physiology section III, vol. 1. Washington, DC: American Physiological Society; 1964. p. 699–714.

Chan WW, Chhowalla M, Glotzer S, Gogotsi Y, Hafner JH, Hammond PT, et al. Nanoscience and nanotechnology impacting diverse fields of science, engineering, and medicine. ACS Publications; 2016.

Chemical Computing Group. Molecular operating environment: chemoinformatics and structure based tools for high throughput screening. Montreal; 2013.

Cigánková H, Mikuška P, Hegrová J, Pokorná P, Schwarz J, Krajčovič J. Seasonal variation and sources of elements in urban submicron and fine aerosol in brno, Czech Republic. Aerosol and Air Quality Research, 20;2021.

Darquenne C. Aerosol deposition in health and disease. J Aerosol Med Pulm Drug Deliv. 2012;25(3):140–7.

Dary CC, Georgopoulos PJ, Vallero DA, Tornero-Velez R, Morgan M, Okino M, Dellarco M, Power FW, Blancato JN. Characterizing chemical exposure from biomonitoring data using the exposure related dose estimating model (ERDEM). In: 17th Annual conference of the international society of exposure analysis, Durham, NC. October 17, 2007.

Derelanko M. Risk assessment. In: Derelanko MJ, Hollinger MA, editors. CRC handbook of toxicology. Boca Raton: CRC Press; 1999.

Derelanko MJ. Risk Assessment. In: Derelanko MJ, Auletta CS, editors. Handbook of toxicology. Boca Raton: CRC Press; 2014.

Diamond ML, de Wit CA, Molander S, Scheringer M, Backhaus T, Lohmann R, et al. Exploring the planetary boundary for chemical pollution. Environ Int. 2015;78:8–15.

Dionisio KL, Frame AM, Goldsmith M-R, Wambaugh JF, Liddell A, Cathey T, et al. Exploring consumer exposure pathways and patterns of use for chemicals in the environment. Toxicol Rep. 2015;2:228–37.

Dix DJ, Houck KA, Martin MT, Richard AM, Setzer RW, Kavlock RJ. The ToxCast program for prioritizing toxicity testing of environmental chemicals. Toxicol Sci. 2007;95(1):5–12.

Doblhoff-Dier P, Bachmayer H, Bennett A, Brunius G, Býrki K, Cantley M, et al. Safe biotechnology 10: DNA content of biotechnological process waste. The Safety in Biotechnology Working Party on the European Federation of Biotechnology. Trends Biotechnol. 2000;18:141–6.

Egeghy PP, Vallero DA, Hubal EAC. Exposure-based prioritization of chemicals for risk assessment. Environ Sci Pol. 2011;14(8):950–64.

Egeghy PP, Sheldon LS, Isaacs KK, Özkaynak H, Goldsmith M-R, Wambaugh JF, et al. Computational exposure science: an emerging discipline to support 21st-century risk assessment. Environ Health Perspect (Online). 2016;124(6):697.

Ehrlich HL. Inorganic hazardous waste amenable to biological transformation. In: Biotechnology for the treatment of hazardous waste. Routledge; 2017. p. 27–44.

Elci SG, Jiang Y, Yan B, Kim ST, Saha K, Moyano DF, et al. Surface charge controls the suborgan biodistributions of gold nanoparticles. ACS Nano. 2016;10(5):5536–42.

Environmental Health Analysis Center. PBT profiler methodology. U.S. Environmental Protection Agency; 2012a [2.000]. Available from: http://www.pbtprofiler.net/Methodology.asp

Environmental Health Analysis Center. PBT profiler. 2.000 ed. U.S. Environmental Protection Agency; 2012b.

Ernstoff AS, Fantke P, Csiszar SA, Henderson AD, Chung S, Jolliet O. Multi-pathway exposure modeling of chemicals in cosmetics with application to shampoo. Environ Int. 2016;92:87–96.

European Chemicals Agency (ECHA). Understanding REACH. 2014.

European Union. Opinion on synthetic biology II-risk assessment methodologies and safety aspects. In: Scientific Committee on Health and Environmental Risks SCoEaNIHR, and Scientific Committe on Consumer Safety, editor.: European Commission; 2015.

Falkner R, Jaspers N. Regulating nanotechnologies: risk, uncertainty and the global governance gap. Glob Environ Polit. 2012;12(1):30–55.

Final Rule. Procedures for chemical risk evaluation under the Amended Toxic Substances Control Act; 2018.

Fröhlich E, Salar-Behzadi S. Toxicological assessment of inhaled nanoparticles: role of in vivo, ex vivo, in vitro, and in silico studies. Int J Mol Sci. 2014;15(3):4795–822.

Gangwal S, Reif DM, Mosher S, Egeghy PP, Wambaugh JF, Judson RS, et al. Incorporating exposure information into the toxicological prioritization index decision support framework. Sci Total Environ. 2012;435:316–25.

Gauthier AM, Fung M, Panko J, Kingsbury T, Perez AL, Hitchcock K, et al. Chemical assessment state of the science: evaluation of 32 decision-support tools used to screen and prioritize chemicals. Integr Environ Assess Manag. 2015;11(2):242–55.

George SC, Hlastala MP. Airway gas exchange and exhaled biomarkers. Compr Physiol. 2011. 1(4):1837–59.

Grieger KD, Hansen SF, Baun A. The known unknowns of nanomaterials: describing and characterizing uncertainty within environmental, health and safety risks. Nanotoxicology. 2009;3(3):222–33.

Grotberg JB. Respiratory fluid mechanics. Phys Fluids (1994–present). 2011;23(2):021301.

Gustavsson MB, Hellohf A, Backhaus T. Evaluating the environmental hazard of industrial chemicals from data collected during the REACH registration process. Sci Total Environ. 2017;586:658–65.

Guy A, Gauthier C, Griffin G, editors. Adopting alternative methods for regulatory testing in Canada. Proceedings of the 6th world congress on alternatives & animal use in the life sciences AATEX; 2008.

Hamraz B, Caldwell NH, Clarkson PJ. A multidomain engineering change propagation model to support uncertainty reduction and risk management in design. J Mech Des. 2012;134(10):100905.

Harder R, Holmquist H, Molander S, Svanström M, Peters GM. Review of environmental assessment case studies blending elements of risk assessment and life cycle assessment. Environ Sci Technol. 2015;49(22):13083–93.

Harremoës P, Gee D, MacGarvin M, Stirling A, Keys J, Wynne B, et al. Late lessons from early warnings: the precautionary principle 1896–2000. Office for Official Publications of the European Communities; 2001.

Heath JR. Nanotechnologies for biomedical science and translational medicine. Proc Natl Acad Sci. 2015;112(47):14436–43.

Hilton DC, Jones RS, Sjödin A. A method for rapid, non-targeted screening for environmental contaminants in household dust. J Chromatogr A. 2010;1217(44):6851–6.

Hubal EAC, Richard AM, Shah I, Gallagher J, Kavlock R, Blancato J, et al. Exposure science and the US EPA National Center for Computational Toxicology. J Expo Sci Environ Epidemiol. 2010;20(3):231–6.

International Commission on Radiological Protection Task Force on Lung Dynamics, Task Group on Lung Dynamics. Deposition and retention models for internal dosimetry of the human respiratory tract. Health Phys. 1966;12(2):173.

Isaacs K, Rosati J, Martonen T, Ruzer L, Harley N. Modeling deposition of inhaled particles. In: Aerosols handbook. Boca Raton: CRC Press; 2012. p. 83–128.

Issacs KK, Rosati JA, Martonen TB. Modeling deposition of inhaled particles. In: Aerosols handbook: measurement, dosimetry, and health effects. Boca Raton: CRC Press; 2012.

Ivask A, Mitchell AJ, Malysheva A, Voelcker NH, Lombi E. Methodologies and approaches for the analysis of cell–nanoparticle interactions. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2018;10(3):e1486.

Jain KK. Nanobiotechnology and personalized medicine. In: The handbook of nanomedicine. New York: Springer; 2017. p. 569–74.

Jain PK, Gupta VK, Bajpai V. Recent advances in environmental biotechnology. LAP Lambert Academic Publishing, Republic of Moldova, Chisinau-2068, str. A.Russo 15, of.61. 2011. ISBN: 978-3-8443-0687-3.

Jin X, Zha J, Xu Y, Giesy JP, Richardson KL, Wang Z. Derivation of predicted no effect concentrations (PNEC) for 2, 4, 6-trichlorophenol based on Chinese resident species. Chemosphere. 2012;86(1):17–23.

Judson RS, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Mortensen HM, et al. In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environ Health Perspect (Online). 2010;118(4):485.

Judson RS, Kavlock RJ, Setzer RW, Cohen Hubal EA, Martin MT, Knudsen TB, et al. Estimating toxicity-related biological pathway altering doses for high-throughput chemical risk assessment. Chem Res Toxicol. 2011;24(4):451–62.

Karmaus AL, Filer DL, Martin MT, Houck KA. Evaluation of food-relevant chemicals in the ToxCast high-throughput screening program. Food Chem Toxicol. 2016;92:188–96.

Kerr A. Dead space ventilation in normal children and children with obstructive airways diease. Thorax. 1976;31(1):63–9.

Klaine SJ, Alvarez PJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, et al. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem. 2008;27(9):1825–51.

Kortelainen M. The REACH authorisation procedure–follow-up and prediction as a downstream user. 2015.

Kortenkamp A, Backhaus T, Faust M. State of the art report on mixture toxicity. Contract. 2009;70307(2007485103):94–103.

Landsiedel R, Ma-Hock L, Kroll A, Hahn D, Schnekenburger J, Wiench K, et al. Testing metal-oxide nanomaterials for human safety. Adv Mater. 2010;22(24):2601–27.

Larese FF, D'Agostin F, Crosera M, Adami G, Renzi N, Bovenzi M, et al. Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicology. 2009;255(1–2):33–7.

Lead JR, Batley GE, Alvarez PJ, Croteau MN, Handy RD, McLaughlin MJ, et al. Nanomaterials in the environment: behavior, fate, bioavailability, and effects—an updated review. Environ Toxicol Chem. 2018;37(8):2029–63.

Li S-D, Huang L. Pharmacokinetics and biodistribution of nanoparticles. Mol Pharm. 2008;5(4):496–504.

Li X, Lenhart JJ, Walker HW. Aggregation kinetics and dissolution of coated silver nanoparticles. Langmuir. 2011;28(2):1095–104.

Linkov I, Satterstrom F, Kiker G, Batchelor C, Bridges T, Ferguson E. From comparative risk assessment to multi-criteria decision analysis and adaptive management: recent developments and applications. Environ Int. 2006;32(8):1072–93.

Liu R, Zhao D. Reducing leachability and bioaccessibility of lead in soils using a new class of stabilized iron phosphate nanoparticles. Water Res. 2007;41(12):2491–502.

Loehr R, Goldstein B, Nerode A, Risser P. Safeguarding the future: credible science, credible decisions. The report of the expert panel on the role of science at EPA. Washington, DC: Environmental Protection Agency; 1992.

Long Y, Long Y-Y, Liu H-C, Shen D-S. Degradation of refuse in hybrid bioreactor landfill. Biomed Environ Sci. 2009;22(4):303–10.

MacCuspie RI, Rogers K, Patra M, Suo Z, Allen AJ, Martin MN, et al. Challenges for physical characterization of silver nanoparticles under pristine and environmentally relevant conditions. J Environ Monit. 2011;13(5):1212–26.

Mackay D, Fraser A. Bioaccumulation of persistent organic chemicals: mechanisms and models. Environ Pollut. 2000;110(3):375–91.

Mansour F, Al-Hindi M, Saad W, Salam D. Environmental risk analysis and prioritization of pharmaceuticals in a developing world context. Sci Total Environ. 2016;557:31–43.

McKone T, Riley W, Maddalena R, Rosenbaum R, Vallero D. Common issues in human and ecosystem exposure assessment: the significance of partitioning, kinetics, and uptake at biological exchange surfaces. Epidemiology. 2006;17(6):S134.

Modeling NM. Applications in bioremediation of hydrocarbon pollutants. In: Microbial action on hydrocarbons. Singapore: Springer; 2018. p. 181–97.

Moya J, Phillips L, Schuda L, Wood P, Diaz A, Lee R, et al. Exposure factors handbook: 2011 edition. Washington, DC: US Environmental Protection Agency; 2011.

Muñoz R, Villaverde S, Guieysse B, Revah S. Two-phase partitioning bioreactors for treatment of volatile organic compounds. Biotechnol Adv. 2007;25(4):410–22.

Nanotechnology Industries Association. Chemicals & raw materials 2019. Available from: http://www.nanotechia.org/sectors/chemicals-raw-materials
National Research Council. Risk assessment in the federal government: managing the process. Committee on the Institutional Means for Assessment of Risks to Public Health, editor. Washington, DC: National Academy of Sciences; 1983.
National Research Council. Biosolids applied to land: advancing standards and practices. Washington, DC, National Academies Press; 2002.
National Research Council. Toxicity testing in the 21st century: a vision and a strategy. Agents CoTTAoE, editor. National Academies Press; 2007.
National Research Council. Science and decisions: advancing risk assessment. Washington, DC: The National Academies Press; 2009a. 424 p
National Research Council. Science and decisions: advancing risk assessment. Washington, DC: National Academy Press; 2009b.
National Research Council. Exposure science in the 21st century: a vision and a strategy. Washington, DC: The National Academies Press; 2012. 196 p
Okabe H, Splitstone PL, Ball JJ. Ambient and source SO2 detector based on a fluorescence method. J Air Pollut Control Assoc. 1973;23(6):514–6.
Persson E. What are the core ideas behind the precautionary principle? Sci Total Environ. 2016;557:134–41.
Pieters M, Kramer H, Slob W. Evaluation of the uncertainty factor for subchronic-to-chronic extrapolation: statistical analysis of toxicity data. Regul Toxicol Pharmacol. 1998;27(2):108–11.
Poel I. The introduction of nanotechnology as a societal experiment. In: Technoscience in progress managing the uncertainty of nanotechnology. Amsterdam: IOS Press; 2009. p. 129.
Poynton HC, Lazorchak JM, Impellitteri CA, Smith ME, Rogers K, Patra M, et al. Differential gene expression in Daphnia magna suggests distinct modes of action and bioavailability for ZnO nanoparticles and Zn ions. Environ Sci Technol. 2010;45(2):762–8.
Poynton HC, Lazorchak JM, Impellitteri CA, Blalock BJ, Rogers K, Allen HJ, et al. Toxicogenomic responses of nanotoxicity in Daphnia magna exposed to silver nitrate and coated silver nanoparticles. Environ Sci Technol. 2012;46(11):6288–96.
Puzyn T, Rasulev B, Gajewicz A, Hu X, Dasari TP, Michalkova A, et al. Using nano-QSAR to predict the cytotoxicity of metal oxide nanoparticles. Nat Nanotechnol. 2011;6(3):175.
Rahola T, Hattula T, Korolainen A, Miettinen J. Elimination of free and protein-bound ionic mercury (203 Hg 2+) in man. Ann Clin Res. 1973;5(4):214–9.
Rogers KR, Bradham K, Tolaymat T, Thomas DJ, Hartmann T, Ma L, et al. Alterations in physical state of silver nanoparticles exposed to synthetic human stomach fluid. Sci Total Environ. 2012;420:334–9.
Rubow KL, Stange LL, Huang B, editors. Advances in filtration technology using sintered metal filters. 3rd China international filtration exhibition and conference; 2004.
Ruckelshaus WD. Science, risk, and public policy. Science. 1983;221(4615):1026–8.
Sahlin U. Uncertainty in QSAR predictions. Altern Lab Anim. 2013;41(1):111–25.
Sahlin U, Jeliazkova N, Öberg T. Applicability domain dependent predictive uncertainty in QSAR regressions. Mol Informatics. 2014;33(1):26–35.
Sample JL, Charles HK Jr. Systems engineering at the nanoscale. J Hopkins APL Tech Dig. 2012;31(1):50–7.
Satterfield T, Kandlikar M, Beaudrie CE, Conti J, Harthorn BH. Anticipating the perceived risk of nanotechnologies. Nat Nanotechnol. 2009;4(11):752.
Schulte PA, McKernan LT, Heidel DS, Okun AH, Dotson GS, Lentz TJ, et al. Occupational safety and health, green chemistry, and sustainability: a review of areas of convergence. Environ Health. 2013;12(1):1.
Schurch S, Lee M, Gehr P. Pulmonary surfactant: surface properties and function of alveolar and airway surfactant. Pure Appl Chem. 1992;64(11):1745–50.
Science & Environmental Health Network, editor. Wingspread conference on the precautionary principle 1998.

Sekine R, Khurana K, Vasilev K, Lombi E, Donner E. Quantifying the adsorption of ionic silver and functionalized nanoparticles during ecotoxicity testing: test container effects and recommendations. Nanotoxicology. 2015;9(8):1005–12.
Shanker AK. 21 Mode of action and toxicity of trace elements. 2008.
Sims JG, Steevens JA. The role of metabolism in the toxicity of 2, 4, 6-trinitrotoluene and its degradation products to the aquatic amphipod Hyalella azteca. Ecotoxicol Environ Saf. 2008;70(1):38–46.
Singh C. 19_The precautionary principle and environment protection. 2016.
Solomon JD, Vallero DA. From our partners – communicating risk and resiliency: special considerations for rare events. Center for Infrastructure Protection & Homeland Security, George Mason University; 2016. Available from: https://cip.gmu.edu/2016/06/01/partners-communicating-risk-resiliency-special-considerations-rare-events/
Stone V, Miller MR, Clift MJ, Elder A, Mills NL, Møller P, et al. Nanomaterials versus ambient ultrafine particles: an opportunity to exchange toxicology knowledge. Environ Health Perspect. 2017;125(10):106002.
Toro D, Hellweger F. Long-range transport and deposition: the role of Henry's law constant. Final report. International Council of Chemical Associations; 1999.
Turvey CG, Mojduszka EM, Pray CE. The precautionary principle, the law of unintended consequences, and biotechnology agricultural biotechnology: ten years after; July 6–10, 2005. Ravello: International Consortium on Agricultural Biotechnology Research (ICABR); 2005.
U.S. Environmental Protection Agency. An examination of EPA risk assessment principles and practices. Washington, DC: US Environmental Protection Agency; 2004a.
U.S. Environmental Protection Agency. Air quality criteria for particulate matter. USEPA; 2004b.
U.S. Environmental Protection Agency. Landfill bioreactor performance: second interim report: outer loop recycling & disposal facility – Louisville, Kentucky. In: Laboratory NRMR, editor. Cincinnati: USEPA 2007.
U.S. Environmental Protection Agency. Endocrine disruptor screening program (EDSP) overview 2017a [updated February 22, 2017. Available from: https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-overview
U.S. Environmental Protection Agency. Guidelines for human exposure assessment: Peer review draft. In: Forum RA, editor. Washington, DC: U.S. EPA; 2017b.
UNEP. Regionally based assessment of persistent toxic chemicals. Global report. United Nations Environment Programme, Chemicals Division; 2003.
United Nations Environment Programme. Rio declaration on environment and development. Nairobi, Kenya; 1992.
US Environmental Protection Agency. Framework for ecological risk assessment. Washington, DC: USEPA Risk Assessment Forum. EPA 630/R-92/001; 1992.
Vallero DA. Fundamentals of air pollution. 4th ed. Amsterdam/Boston: Elsevier; 2008. xxiii, 942 pp.
Vallero D. Environmental biotechnology: a biosystems approach. Amsterdam: Academic; 2010a.
Vallero D. Environmental contaminants: assessment and control. Academic; 2010b.
Vallero D. Nano-cerium fuel additives phase distribution. In: Weinstein J, editor. Research Triangle Park: U.S. Environmental Protection Agency, National Exposure Research Laboratory; 2012.
Vallero DA. Measurements in environmental engineering. In: Kutz M, editor. Handbook of measurement in science and engineering. Hoboken: Wiley; 2013.
Vallero DA. Fundamentals of air pollution. 5th ed. Waltham: Elsevier Academic Press; 2014. 999 pages cm p.
Vallero D. Environmental biotechnology: a Biosystems approach. San Diego: Elsevier Science; 2015.
Vallero DA. Air pollution monitoring changes to accompany the transition from a control to a systems focus. Sustainability. 2016;8(12):1216.
Veldhuizen EJ, Haagsman HP. Role of pulmonary surfactant components in surface film formation and dynamics. Biochim Biophys Acta (BBA) Biomembr. 2000;1467(2):255–70.

Wambaugh JF, Setzer RW, Reif DM, Gangwal S, Mitchell-Blackwood J, Arnot JA, et al. High-throughput models for exposure-based chemical prioritization in the ExpoCast project. Environ Sci Technol. 2013;47(15):8479–88.

Wambaugh JF, Wang A, Dionisio KL, Frame A, Egeghy P, Judson R, et al. High throughput heuristics for prioritizing human exposure to environmental chemicals. Environ Sci Technol. 2014;48(21):12760–7.

Warheit DB. Nanoparticles: health impacts? Mater Today. 2004;7(2):32–5.

Wiesner MR, Lowry GV, Jones KL, Hochella J, Michael F, Di Giulio RT, Casman E, et al. Decreasing uncertainties in assessing environmental exposure, risk, and ecological implications of nanomaterials†‡. Environ Sci Technol. 2009;43(17):6458–62.

Wild CP. The exposome: from concept to utility, *International Journal of Epidemiology*, 2012;41(1):24–32. https://doi.org/10.1093/ije/dyr236

Williams DP, Park BK. Idiosyncratic toxicity: the role of toxicophores and bioactivation. Drug Discov Today. 2003;8(22):1044–50.

Wood MD, Plourde K, Larkin S, Egeghy PP, Williams AJ, Zemba V, ... Vallero DA. Advances on a dcision aalytic aproach to eposure-bsed cemical pioritization. Risk Analysis, 2020;40(1);83–96.

World Health Organization. Environmental health criteria 214: Human exposure assessment. International Programme on Chemical Safety. 2000.

Zhang X, Arnot JA, Wania F. Model for screening-level assessment of near-field human exposure to neutral organic chemicals released indoors. Environ Sci Technol. 2014;48(20):12312–9.

Nanotoxicology and Risk Perception among Public and Elite Groups

Barbara Herr Harthorn, Terre Satterfield, and Nick Pidgeon

Contents

Abstract In this chapter, we provide an overview of an unprecedented body of new knowledge about the emergence of perceived risks and benefits of nanotechnologies and selected other new technologies through a set of linked studies. The chapter highlights the results of over a decade of mixed methods social science research at the Center for Nanotechnology in Society at the University of California at Santa Barbara with reference to other key publications in the field. The chapter reviews research on: views, perceptions, values, and attitudes and social action among multiple stakeholders in the nanotechnology enterprise; development and refinement of innovative methods for public engagement with new technologies in the US and comparative other nations; experts' risk knowledge and views on regulatory preparedness for safe handling of novel nanomaterials' properties; and print and social media and policy attention focused on nanotech risks and benefits, particularly with reference to emergent public perceptions, risk amplification, or attenuation. In addi-

B. H. Harthorn (✉)
Center for Nanotechnology in Society and Department of Anthropology, University of California at Santa Barbara, Santa Barbara, CA, USA
e-mail: harthorn@ucsb.edu

T. Satterfield
Institute for Resources, Environment and Sustainability, AERL Room 417, 2202 Main Mall, The University of British Columbia, Vancouver, BC, Canada

N. Pidgeon
Understanding Risk Group and Tyndall Centre for Climate Change Research, School of Psychology, Cardiff University, Cardiff, UK

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_7

tion, the chapter details modes of dissemination of such societal knowledge to an array of critical stakeholders, including scientists and engineers developing these new materials and their enabled systems and products, nanotoxicologists who have been assessing the environmental and health risks presented by such novel materials, the international nanomaterials industry, policymakers/regulators, journalists, the diverse US public, and NGOs and civil society organizations (CSOs). Taken together, the portfolio of new knowledge produced, the methodological advancement evident in its production, and the transfer of knowledge accomplished through engagement with diverse multi-stakeholders are argued to constitute an unprecedented advancement of socio-technical integration. The research process has also generated for the first time a robust international community of socio-technical scholars and experts with the skills and experience to advance societal benefits and ethical governance of emerging technologies.

Keywords Nanotechnologies · Risk and benefit perception · Public participation · Technological governance · Toxicology · Mixed methods social research

Introduction

In the earlier years of the concentrated effort to develop nanotechnologies, a core question of foremost importance to many governing bodies, was 'would nanotechnologies be an object of concern'? Would such technologies face significant backlash or stigma from an imagined public? Could such a backlash limit the realization of their potential economic and/or social benefits, despite billions of dollars invested into national innovation systems, including the US National Nanotechnology Initiative (NNI 2019)? This chapter explores these questions, with the benefit of hindsight and the insights gained through the lens of a suite of collaborative, international research projects. Ultimately, understanding the perceived risks and benefits of these emerging technologies is neither simple nor straightforward.[1] We anticipated that risk would come easily to mind amongst study participants given extensive evidence on public and expert views on other new technologies (e.g., see Slovic 2000, 2010; Pidgeon et al. 2003), and we anticipated legitimate concerns regarding job creation or loss, as well as optimism regarding tangible benefits such as adequate health, safety and environmental protection. Concerns regarding regulation, trust, responsibility, and justice were also expected as were questions about acceptable and affordable alternatives, and scientific uncertainty about the risks. Further, we also suspected that context matters, and thus views might vary regarding

[1] The purpose in this chapter is primarily to synthesize the contributions to the study of expert and public perceptions of nanotechnologies and nanotoxicologies by one large interdisciplinary, international team of risk perception researchers. Publications by others in the field are cited as appropriate but are not the main focus. For a complete cumulative list of the CNS-UCSB risk perception and social response group's publications 2006–2016, please see www.cns.ucsb.edu

particular nano-enabled products (glossed here and in many other publications as 'application') and their requisite nanomaterials. We believed that experts should discuss, anticipate, and address these concerns, but we had little idea how an 'early and often' social science approach to understanding risk would play out, nor did we anticipate the upheaval in the fundamental structure of the public communication system produced by the internet over the course of the project. These latter concerns created numerous conceptual and practical challenges for studying the effects of communication about these new technologies on perceptions and their implications for society.

Beginning in 2005 and proceeding through 2016,[2] the 3 co-authors of this chapter jointly led a group at the NSF-funded Center for Nanotechnology in Society at University of California at Santa Barbara (CNS-UCSB). We had the opportunity to conduct a series of linked studies alongside a talented group of colleagues and graduate students on formative nanotech perceived risks and benefits over time. We did so by means of a carefully calibrated and staged set of mixed qualitative and quantitative social science research methods aimed at studying the views and beliefs about these new technologies by *multiple parties*. By 'multiple parties' we mean people in numerous and different social locations including: (1) science and technology (S&T) research and development (R&D) experts, that is, scientists and engineers working at the nanoscale; (2) nanotechnology risk assessment experts, regulators and government agency personnel, (3) industry leaders, scientists, safety personnel and workers, (4) nongovernmental organizations (NGOs) or other social movement organizations (SMO) and special interest groups, and science journalists, and (5) members of the public who differ by gender, race/ethnicity, class, occupation, education, and age, and many other characteristics, as well as nationality. An important aspect of our work has been a shared interest in investigating the diversity and nuances of views both within and across these categories of difference. We have pursued this interest because of the demonstrated importance of democratic participation in the innovation system (Dietz and Stern 2008), the ethical imperatives of responsible research and innovation (Rip et al. 1995; Guston and Sarewitz 2002; Owen et al. 2013), and the challenges to full participation posed by a large and complex multicultural society such as the US. In addition, we proposed to implement deliberative models for enhanced public participation in technological dialogue—methods developed primarily in the UK and therefore new to US studies at that time (e.g., Grove-White et al. 1997; Macnaghten 2004; Pidgeon and Rogers-Hayden 2007; Horlick-Jones et al. 2007; Bickerstaff et al. 2008) and Europe (Hagendijk and Irwin 2006; Kurath and Gisler 2009).

The work in the CNS at UCSB was supplemented by the authors' collaborative association with another large science center, the US NSF- and EPA-funded UC Center for Environmental Implications of Nanotechnology (UC CEIN), based at UCLA with a significant component at UC Santa Barbara (see UCCEIN 2019).

[2] Some collaborative work continues beyond the 2016 sunset of the CNS to the present. There was a very large body of data collected in the multiple studies conducted by the group, and data analysis and dissemination will continue for some time.

Harthorn, Satterfield, Kandlikar and Beaudrie conducted joint expert and public risk perception research in conjunction with the UC CEIN. This complemented and extended that in the CNS, in particular allowing us to conduct expert workshops and studies (Beaudrie et al. 2011, 2013b, 2014), studies of regulatory sufficiency for nanomaterials oversight (Beaudrie 2010; Beaudrie and Kandlikar 2011; Beaudrie et al. 2013a), industry surveys of the international engineered nanomaterials (ENMs) industry (Conti et al. 2008; Engeman et al. 2012, 2013), and environmental risk perception research among diverse cross-sections of the US public (Satterfield et al. 2012, 2018). Harthorn served on the UC CEIN executive committee since its inception in 2008 and as such has been part of numerous expert discussions about the environmental hazards and safety of ENMs (e.g., Holden et al. 2016).

In sum, our overarching goals have been to generate an unprecedented body of new knowledge about the emergence of perceived risks and benefits of nanotechnologies and selected other new technologies through a set of linked studies. The scope of the work has included:

- Studying views and social action among multiple stakeholders in the nano-enterprise;
- Developing and documenting methods for public engagement with new technologies in the US and comparative other sites;
- Characterizing expert knowledge and regulatory preparedness for safe handling of these novel properties;
- Tracking media and policy attention paid to nanotech risks and benefits to provide critical evidence of risk signal amplification or attenuation; and
- Disseminating the knowledge gained to an array of critical stakeholders, including scientists and engineers developing these new materials and their enabled systems and products, nanotoxicologists assessing the environmental and health risks they present, the nanomaterials industry, policymakers/ regulators, journalists, the diverse US public, and NGOs and civil society organizations (CSOs).

Never before has a large class of new technologies anywhere in the world been the focus of such a systematic, long-term, comparative, multi-stakeholder analysis of perceived risk and the societal implications of new technologies. This unique opportunity was realized through US NSF funding, and the considerable additional leverage funding this generated (particularly the aforementioned collaboration with UC CEIN), and the creation of both CNS-UCSB and an engaged, mixed methods research team.

Approach and Methods

The main theoretical framework for this suite of research projects at inception of the CNS in 2006 derived from the Social Amplification of Risk Framework (e.g., Kasperson and Kasperson 1996; Pidgeon et al. 2003), which provides a broad, multi-factorial approach to understanding the evolution of past risk controversies.

For example, changing public and regulatory views on nuclear power have been exhaustively studied by risk analysts across its highly benefit-centric period of strong public support, through to near absolute stigma of these energy systems following the partial meltdown at the Three Mile Island nuclear power plant in 1979 (e.g., Erikson 1994), and thereafter in reference to today's cross-national variance in public support for and opposition to nuclear power (OECD 2010), shaped also by the aftermath of Fukushima Dai-ichi nuclear accident in 2011 (Butler et al. 2011; Pidgeon 2011).

Briefly stated, our work has demonstrated (Satterfield et al. 2009; and below), that nanotech R&D has evolved to the present in the US and abroad with only modest evidence of public awareness, risk aversion, media attention, or widespread protest. As a result, our research moved progressively into methodological experimental research modes to understand these upstream and muted perceptions, even though many of the technologies themselves continue to move downstream into wider commercial production and dissemination. This unprecedented lengthy opportunity to study emergent attitudes, beliefs and perceptions has brought unique research challenges as well. As the work progressed in the absence of once-anticipated risk amplification, a shift in focus to comparisons with other emerging technologies enabled a better understanding of nanotechnologies' reception.

The term 'risk perception' as we are using it here references *cognitive and affective components of risk, which are dynamic and produced through complex drivers*. It includes linked cognitive concepts such as mental maps or models (Morgan et al. 2001) and schemata or templates (Casson 1983) but it also focuses on affective responses that are particularly important in 'fast thinking' intuitive responses where knowledge is low. For example, in the context of survey research, risk perception also references deeper cultural values and beliefs that often underpin survey responses but are better probed in systematic qualitative research, especially in an upstream emerging technology context. Risk perception research overlaps with but is not the same as public opinion or attitude polls and surveys. In particular, risk perception research has shown that public perceptions are influenced by a wide array of psychological and social factors that public opinion polls rarely examine (Slovic 2000; Leiserowitz 2006).

In spite of a rich body of comparative literature on perceived risks (particularly those of the diverse US public) regarding an array of past technologies, the case of nanotechnologies[3] is different in some crucial respects. As indicated above, it has been typified by unusually low public awareness, necessitating the move in our research to what is best understood as 'far upstream.' Studying public attitudes and risk/benefit judgment formation in progress—as they take shape and are produced—greatly extends the analytic terrain of attenuated (limited) risk perception. We thus asked more fundamental questions about how people make sense of novel technologies in the context of many unknowns and in some cases unimaginable

[3] Due to the large diversity of materials and applications encompassed by the term 'nanotechnology' we prefer in general to follow the recommendations in the UK Royal Society report on Nanoscience and Nanotechnologies (Royal Society 2004) and refer to them in the plural.

characteristics and implications. Low awareness has necessitated particularly delicate approaches to how the research and the technologies are framed.

This upstream world (or moment) has also pushed us to consider what comparatively little is known about anticipated *benefit perception* compared to *risk perception*, and what nanotechnologies' perceived benefits across different sectors of the population signify. For instance, do varied members of the public have ready-to-go *templates* for making cognitive sense of this new unknown terrain or are they creating them anew? Nanotechnologies emerged in the social and imaginative realm as largely inchoate risk objects, indeed as a kind of *tabula rasa* risk object(s). Their ubiquity, invisibility and uncertainty suggest consideration of what Morton (2013) has referred to as "hyperobjects," described as "entities of such vast temporal and spatial dimensions that they defeat traditional ideas about what a thing is." *The combination of ubiquity and invisibility of nanotechnologies, along with the complex global/societal contours that mark their development and deployment, challenge risk perception research in entirely unprecedented ways.* In addition, the social and political contexts of these molecular sized technologies are complicated by experts whose own judgments of risk and benefit and need for regulation are highly uncertain, particularly regarding longer term, downstream implications and consequences of different nanotechnologies. Together these challenges necessitated creation of a new set of research questions as well as a departure from the usual defaults as to what constitutes risk perception research.

Methods

Each of these complications and admittedly spirited challenges has, along the way, compelled us to ask a series of thorny methodological questions. What methodological innovations are needed to capture and understand public engagement and thinking as it is unfolding rather than the conventional downstream risk controversy approach where judgments are vastly more solidified, if not polarized? Low public awareness creates particular demands for sensitive framing of risk versus benefit information. Upstream deliberation has been essential to providing in-depth qualitative data about emergent ideas, values and beliefs. Cross-cultural implementation has required a more thoughtful approach to protocol development and refinement, and critical reflection on researcher-driven effects is essential at every step. We have used a broad set of systematic qualitative and quantitative methods to address these issues, often starting from in-depth, qualitative methods such as open-ended inductive interviews and group discussions to learn more about the mental models (Morgan et al. 2001) or cognitive maps or schemata (Casson 1983) that people use to think and talk about technologies. We then use this derived knowledge to build quantitative survey instruments to ask well-grounded questions in a systematic and carefully sequenced way, controlling for primacy effects, of much larger and more representative samples.

In essence, we have sought to build a suite of tools where none existed before. Such innovation has included piloting and implementing novel decision pathway survey methods. These enable a more dialogic and iterative approach to engaging larger and more diverse samples than intensive qualitative deliberative work can yet handle (Gregory et al. 2016). At every stage of work, we also sought to carefully theorize and test ever greater use of tutorials and framing styles in surveys, interview protocols, and the elicitation protocols used in deliberative workshops. We did this systematically by varying both information content and opportunities for information seeking (e.g., breaks in deliberation provided for café-style information seeking across a broad array of sources), changing the very format and assumption of survey design (e.g., embedding tutorials, using narrative framings so that new information was more readily comprehensible), and altering the order of risk versus benefit information to assess primacy effects, among other innovations.

Understanding expert judgments as they emerged is also more methodologically challenging in the upstream moment. We conducted this work, that is, early in innovation and development, in conditions of low public awareness and high scientific uncertainty about both the commercial potentials and attendant hazards. Thus, this too was a key focus of our work (Pidgeon and Rogers-Hayden 2007; Rogers-Hayden and Pidgeon 2008). Experts, as with all, are subject to uncertainty in their views on the risks and benefits of the materials and their nano-enabled products; and nanotechnologies represent an extremely large and variable class of materials and processes. Full characterization and standardization of these are still in their early stages 16 years into the process. Although in some other parts of the world precautionary approaches have been implemented, for example the REACH program in the EU (REACH 2019), and NICNAS's industrial nanomaterial and nanotechnology regulation in Australia (NICNAS 2019), engineered nanomaterials (ENMs) are not yet subject to special regulatory controls in the US beyond individual company- and material-specific products under TSCA, and regulatory gaps are considerable (see Beaudrie 2010; Beaudrie et al. 2013a). Methodological approaches in this still malleable context have included in-depth interviews with elite nanoscientists (Harthorn and Mohr 2012b), survey research across different communities of experts to capture affiliation-based variance (Beaudrie et al. 2013b; Beaudrie et al. 2014), and expert workshops designed to develop decision tools to bridge both uncertainty and regulatory gaps (Beaudrie and Kandlikar 2011; Beaudrie et al. 2011, 2015).

Together this suite of efforts included standard, psychometric, consumer, and experimental decision pathway phone and web-based surveys of demographically diverse and representative US (and other) members of the public. But also surveyed was a range of experts including scientists and engineers, regulators, and industry leaders, and NGOs were systematically studied as well. Experimental research was conducted on factors driving group polarization in emerging nanotechnology debate, as was longitudinal tracking of print and internet media coverage of nanotechnologies, and longitudinal tracking and analysis of citizen mobilization and action around nanotech products, research, and development. We also employed systematic qualitative research methods that provide a substantive basis for and

validation of quantitative results and include mental models interviewing, expert interviews, expert structured decision-making workshops, ethnographic interviews, and deliberative public engagement workshops and focus groups regarding the risks and benefits of specific applications of nanotechnologies and related new technologies. In all research, a focus on the effects of application domains on perceived benefits and risks was also key, whether environmental or 'green nano,' energy-efficient technologies, medical innovations, or military innovations.

Together, this research was designed to comprehensively examine the *situated knowledge, perceptions, and beliefs* of the main actors in the nano-enterprise. By "situated knowledge" we draw on social theory to indicate that knowledge (and imagination) are both shaped and conditioned (but not necessarily determined) by social location and position, and that social values, perception and knowledge production are socially organized and co-produced through dialogue (Stoetzler and Yuval-Davis 2002). These ideas and the broader social theoretical foundations of the work are examined extensively in Harthorn and Mohr (2012a, b) and Harthorn (2017a).

Lastly, our collaborations and research—with their focus on expert and public risk perceptions—have to some extent bridged the strong focus in the NNI (2019) on EHS (environmental health and safety) issues and the more modest focus on societal (ELSI—ethical, legal, and social issues). This disparity is evident in the relative funding allocated to these areas of research throughout the history of the NNI (see Harthorn 2017b, 1544). Yet, the focus on risk perception, a vital component of valid risk communication, has arguably increased the reach of our work (see Pidgeon et al. 2011a).

The research reported below addresses these many issues. We have organized our discussion into three main foci: (1) the "problem" of public acceptance; (2) the regulatory challenges of nanotech; and (3) engaging the public: from precaution to responsible research and innovation.

Main Findings

The "Problem" of Public Acceptability

Background. Government, industry, and scientists often express concern about presumed lack of public acceptability as a major potential impediment to technological development. This has been the case throughout the development of nanotechnologies, and yet what constitutes acceptability is not as straightforward as it first appears (cf. Devine-Wright 2007; Demski 2011). In the low knowledge context of emerging technologies, we have found that diverse groups of the public are often uncertain rather than assertive or habitually inclined toward risk-averse stances. For example, in our meta-analysis of all nanotech public attitude surveys in North America, Europe and Japan prior to 2009, on average almost half, or 44% of

respondents were very knowledge and risk uncertain – that is, they replied that they "don't know" or are "not sure" about whether the risks outweigh the benefits or the benefits outweigh the risks (Satterfield et al. 2009). This low knowledge context or lack of familiarity thus suggested that preferences were still largely unformed for good reasons, necessitating a specific thinking about the qualities and conditions of new technology production. That is, risk perception and technological preferences are appropriately conditional, however much some evidence for the role of other variables (such as negative affect or distrust) is also present.

Another deceptively simple question is who are nanotechnologies' diverse public participants? An early meeting convened by the US National Nanotechnology Coordination Office (NNCO) in 2006 struggled to address this issue in the low awareness context, and many others since then have dealt with 'the public' as 'stakeholders' in very different ways. Some are invited to participate (and speak for the wider and more diverse public). For example, NISEnet and nano science education approaches have defined 'the public' as the science-interested (and presumably knowledge deficit-ridden) public who seek out and attend science museums and other science education events, including the Nano Days events we convened annually in the Santa Barbara community from 2006 to 2016. Survey researchers, ourselves included, have used representative national samples (and quota samples thereof), in the US and elsewhere, to gather and to some extent speak for those diverse but anonymous people. Our deliberative work has used a similar (but necessarily smaller scale) logic to draw quasi-representative diverse quota samples from the communities in which the deliberations are held.

From a *normative ethics* point of view, the relevant members of the public are those who might be affected by the development, and so follow the ethic of informed consent (however contingent). But with such ubiquitous technologies or hyperobjects, that is virtually everyone, a universe we have no means to directly and fully engage. Thus, the above means have served as proxies. From an *instrumental* point of view, those members of the public who may be most strategic to understand and engage with, particularly for a governing body whose mandate includes public acceptance, are those who are most concerned (and vociferous). In the nanotech case that has been a set of key social movement organizations (SMOs)/civil society organizations (CSOs), glossed here as non-governmental organizations (NGOs). For this reason and for their importance as actors in the Social Amplification of Risk Framework (Pidgeon et al. 2003), we have mapped the actions of the full English-language NGO nano-active population over the past decade, looking closely at the views of watchdog organizations interested in particular risk scenarios (Engeman and Harthorn 2013; Engeman et al. 2017).

The shifting sands of science journalism, print media, and social media have provided a dynamic and challenging context for research on media coverage and its potential effects on public views on nanotechnologies. We have conducted three sequential print and social media projects led at UCSB by Bruce Bimber (2006–2010), at Lehigh University by Sharon Friedman (2010–2015), and at UCSB by Ariel Hasell (2014–2016) with Galen Stocking. The Bimber-led project at CNS was based on 10 years of nanotech news coverage at the top 10 leading print media

outlets in the US in English 1999–2009 and concluded that media coverage of nanotech was quite low overall and episodic compared to other issues, peaked in 2006 in spite of regulatory action and buildup, and that frame analysis (Weaver et al. 2009) showed that like other science journalism, ideas about progress dominated (70% of the nano news as a whole was on progress), while the news on the social implications of nano displayed progress and risk frames at nearly the same volume. Nanotech domains or applications were distributed differently over that decade, with more concrete applications emphasized over time, and journalists only using the progress frame in relation to nano applications in medicine, energy, computers and economy. Notably, in spite of experts' and regulators' emphasis on nanotechnologies for environmental remediation, the "…connection the media draws between nano and the environment seems to be a story of harm and not benefit" (Lively et al. 2012: 234) (Fig. 1).

Science journalism scholars Friedman and Egolf, who began their work in conjunction with a NIRT project based at UCLA, came under the CNS umbrella in 2010 and continued longitudinal analysis of nanotechnology risks in twenty US and nine UK newspapers 2000–2014. In 2011, as a part of our edited special issue of *Risk Analysis* (Pidgeon et al. 2011b), they also documented the low coverage of nano in both countries and identified three main narratives over time: runaway technology, science-based studies, and regulation, with recurrent discussion of scientific uncertainty in about half of the articles (Friedman and Egolf 2011). The continued decline in coverage of nano in conjunction with the general erosion of science journalism in the US and abroad more broadly challenged traditional media studies approaches (Friedman and Egolf 2012). In 2014–2016, we moved to social media with a project by CNS-UCSB graduate fellows Galen Stocking and Ariel Hasell. The research uses Forsight by Crimson Hexagon to access the content of all publicly available messages posted on Twitter, focusing on messages related to

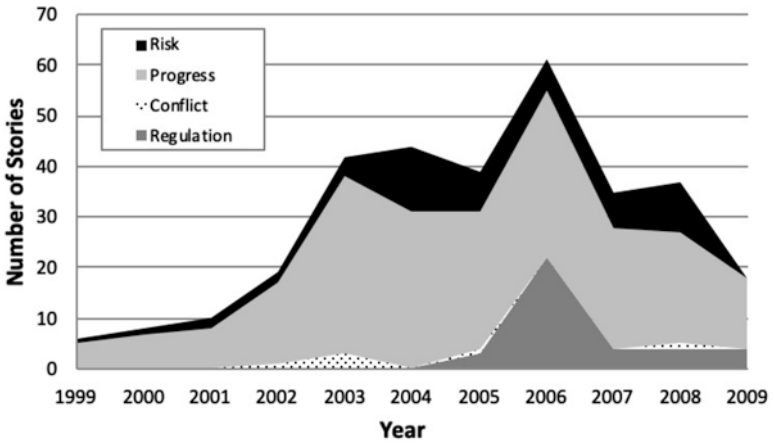


Fig. 1 Frequency of nanotechnology news frames in print media, 1999–2009. (Based on Lively et al. 2012: 234)

Table 1 Gender deliberation study design for 6 workshops by (Harthorn et al. In preparation)

2009 Workshops	Energy/Environment	Health/Enhancement
Women only	1	1
Men only	1	1
Mixed W/M	1	1

emergent technologies (nanotechnology and fracking in particular), and examined how public discussion of risk unfolds in social media. A series of projects examined the public discussion of fracking on Twitter by examining tens of millions of messages shared by Twitter users. This project has looked at what types of risk objects were highlighted in public discussions of nanotechnology and unconventional oil and gas development (or 'fracking'), and they found that much of the discussion of nanotechnology is about innovation rather than risk, while about half of the discussion of fracking is risk related. The team has made a series of conference presentations (e.g., Stocking and Hasell 2014; Hasell and Hodges 2015, and Hasell 2016), and has several manuscripts in preparation for peer-reviewed publications.

All these studies document very low volume coverage of nano risk issues by media, both traditional print media and social media. This parallel finding from over a decade and a half and across traditional and social media lends strong support to the overall media context of low nanotech risk signal amplification, even with rising risk and regulation issues in play.

In what follows we summarize selected key findings from our work on what drives public acceptability of nanotechnologies.

Benefit Matters

Benefit has long been recognized to be a key component of the risk calculus and so also critical to acceptability judgments (Slovic, personal communication, 2007). However, the focus in much risk perception work is on explaining why, retrospectively, risk amplification, technological stigma or harmful attenuation occurred. Such questions are key to understanding health risks that might follow, but this focus has also resulted in surprisingly little attention to benefit judgment itself. Unpacking what is meant by benefit perception turns out to be critical for understanding nanotech risk perceptions, and perhaps for all far upstream and poorly understood new technologies.

Our work has consequently provided extensive evidence of the largely benefit-centric views the US and UK lay public have of nanotechnologies. This effect is powerfully demonstrated in the quantitative meta-analysis of 22 surveys 2002–2009 conducted in N. America, Europe, and Japan and published in *Nature Nanotechnology* (Satterfield et al. 2009), in which approximately 3 times as many respondents judged the benefits to outweigh the risks of nanotechnology as compared to those who thought the risks outweighed the benefits. The more compelling finding in this study, however, we argued was that on average almost half of respondents (44%)

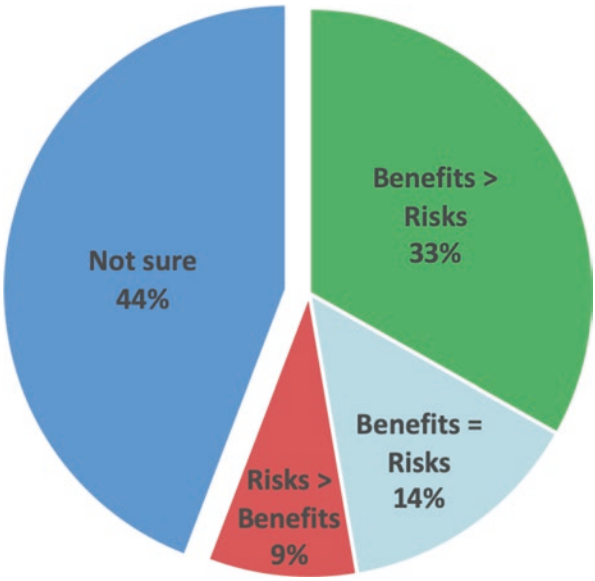


Fig. 2 Public perceptions of nanotechnology risks and benefits: Benefit centric, but high uncertainty and potential malleability, based on 22 surveys 2002–2009 in N. America, Europe & Japan. (Figure by C. Beaudrie data from Satterfield et al. 2009; Benefits > Risks 33.1%; Benefits = Risks 14.1%; Risks > Benefits 8.5%; Not sure 44.1%)

were unsure which was the case. We pointed to this finding's importance for potential future malleability of views in this low awareness, risk sensitive context (Fig. 2).

Our comparative US-UK deliberative work on nanotechnologies also found surprisingly high levels of overall benefit centricity among participants in both countries, a somewhat surprising result given the legacy of interpretive risk 'frames' available from prior controversies in both the UK (e.g., the GMO and BSE, or mad cow, controversies) and US (e.g., high profile chemical contamination disasters, such as at Love Canal). In the case of these deliberations, benefit centricity was predominantly driven by a preoccupation with the technologies themselves, that is, they were assumed to be essentially beneficial until proven otherwise; the sheer novelty of them was often seen in optimistic, even charismatic terms, whereas the risks were overwhelmingly perceived by our participants as social and/or as pertaining to governance demands (Pidgeon et al. 2009).

This analysis is further supported by our experimental survey research in which provision of longer, more detailed narratives about specific technologies, including those with positively-valenced information, did not produce the kind of benefit centricity evident in deliberation work and meta-analyses cited above (Conti et al. 2011). In our 2011 national US web survey, we further found that the benefit centric views were reversible in the face of risk information, which we interpreted as a betrayal effect. That is, presenting risk information (e.g., re: safety concerns about

ENMs) after benefit-only information had a more detrimental effect on risk acceptability than the reverse order (Satterfield et al. 2012).

More recent comparative work we have conducted on shale oil and gas extraction in the US and UK (Thomas et al. 2017; Harthorn et al. 2019). has driven home the degree to which, by contrast, nanotechnologies in these upstream contexts are essentially placeless—we were discussing technologies that in many cases were still on the S&T drawing boards or even just imagined technological futures, rather than material, geospatially situated technologies such as unconventional oil and gas extraction. We conclude that much of nanotechnology's benefit 'halo' derives from this, and will remain there, far from our risk detecting sensorium, until risk 'takes place'—that is, until waterways are polluted, an explosion occurs, a transportation spill happen or in some cases not even then. We note that chemicals more generally are placeless in this way as well, until a facility/use exists (e.g., Irwin et al. 1999; Bush et al. 2001). The critical aspect of this for risk perception is that upstream benefit judgments should not be assumed to be fixed or enduring. Rather, what acceptance there is may well be fragile, and is necessarily contingent, particularly in the nanotech case where awareness is very low and invisibility of the technologies and their footprints is the dominant feature. *Upstream benefit and acceptability ratings may thus be highly mobile, and the nanotechnologies case demonstrates this perfectly.*

Application Matters

We made a strategic decision in 2005 when launching this research initiative not to focus on generic 'nanotechnology' but instead to work with its specific applications. This is in part because our close work with nanoscale scientists and engineers (NSE) indicated that many of them found the generic term problematic or meaningless. In part due to Nick Pidgeon's role, we have in general followed the advice of the UK Royal Society in its report on *Nanoscience and nanotechnologies: opportunities and uncertainties* (Royal Society and the Royal Academic of Engineering 2004). As the title indicates, the Royal Society chose to refer to the plurality of nanotechnologies. In following this, we have assumed from the start that differences between technologies—either the engineering nanomaterials (ENMs) themselves or more complex nano-enabled applications—would be important. And this has indeed been borne out in the findings of our research.

We have followed specific nano-applications throughout our research design of many studies. Both series of nanotechnology deliberations we conducted (2007, US-UK; 2009, US gender) were structured to systematically compare nanotechnology energy applications with nanotechnology medical/health/enhancement applications by convening separate workshops on each application topic. The findings from both sets of studies reveal stark differences in perceived risk by application. The US-UK comparative study found that cross-national differences were dwarfed by strong differences across applications, with unmitigated enthusiasm for energy applications, particularly those emphasizing renewable/new forms of energy rather

than energy conservation technologies like energy efficient lighting. By contrast, medical technologies elicited far more nuanced and ambivalent views in participants in both countries, particularly concerning issues of fairness and distributive justice, responsibility, and in the case of human enhancement technologies, significant moral and ethical concerns (Pidgeon et al. 2009). These application effects between energy and health were even more evident in the 2009 gender deliberations in the US (Harthorn et al. 2012; Rogers et al. 2012), and we found that food and food packaging applications were viewed with universal mistrust and dislike (Rogers-Brown et al. 2011). These three different application domains show the important contextual information diverse members of the public draw on to make sense of both risk and benefit from new technologies. Abundant clean energy is seen as so urgently needed, such a high benefit application, that risks are not even part of the picture. Medical applications themselves are seen as highly beneficial technically, but not necessarily socially acceptable due to likely high cost and restricted access, and also due to potential threats to privacy. And both food and food packaging applications are seen as providing consumers with little to no benefit and possible high risk—they represent technological interference with a perfectly acceptable existing product, while the benefits are seen as accruing solely to the grocery industry. Applications do matter greatly.

Our survey research has also confirmed strong application effects in experimental design protocols. For example, our 2008 US national phone survey was designed to assess how different nanotechnology applications were viewed by those in/from different social positions. We focused on applications of food, health and energy, and we explored in particular how vulnerability and environmental justice concerns affected acceptability of different applications (Conti et al. 2011). We systematically altered information framing—from fully benefit centric to fully risk centric. We found a nanofood application to be highly unacceptable to survey respondents, even in its most positive, all benefit presentation form. Our national phone survey also found strong application effects, in interaction with other safety and contextual variables, with nano-energy and nanotechnology electronic applications seen as highly beneficial, whereas medical and environmental applications were more affected by other contextual variables (Satterfield et al. 2012).

While application has had a noticeable or strong effect on nanotech risk perception in this work, we have somewhat surprisingly found no such effect of the specific type of nanomaterial (ENM). Since carbon nanotubes (CNTs) have been the focus of regulatory action, and regulatory action tends to generate news coverage and risk amplification among other new technologies, we anticipated that there might be more concern about CNTs than other ENMs. However, our 2010 and 2012 national web-based environmental risk perception surveys in which we included ENM types including CNTs as variables provided almost no evidence for effects of the ENM type on public acceptability. This is likely an effect of low awareness and low media attention.

Risk Signal Matters

By contrast, risk signal, the characterization of riskiness, particularly from trusted sources, matters a great deal in diverse groups of the public's risk versus benefit judgments about specific nanotechnologies. We anticipated this in this low awareness/knowledge situation, and therefore we built risk signal into all survey protocols from the start as a test condition. By *risk signal* we mean, *the provision of information that indicates experts attribute 'minimal,' 'moderate,' 'significant,' or 'uncertain' risk*. In our 2010 national web-based survey we found that sensitivity to the risk signal, regardless of the particular application, prevailed. This was true across environmental, medical, energy, and military applications using different ENMs (among 8 types). This "dominance" of risk signal was true and affected judgments of acceptability, even when nanomaterials and applications were carefully described (Harthorn et al. 2011a; Satterfield, Harthorn, Collins, and Pitts In preparation (a)). With one exception (an environmental remediation application), the relative ranking of acceptability of 13 of the 14 applications described in the research protocol is positively correlated with the degree of risk attributed to it in the description participants received. This same figure shows the lack of sensitivity to ENM type (see above) (Fig. 3).

In all our qualitative, deliberative research we also worked strenuously to present technological risks and benefits in as balanced a form as possible, aiming for neutral researcher effects on risk judgments to the highest degree possible. We have expressly *avoided* producing risk amplification (or attenuation) because the aim is

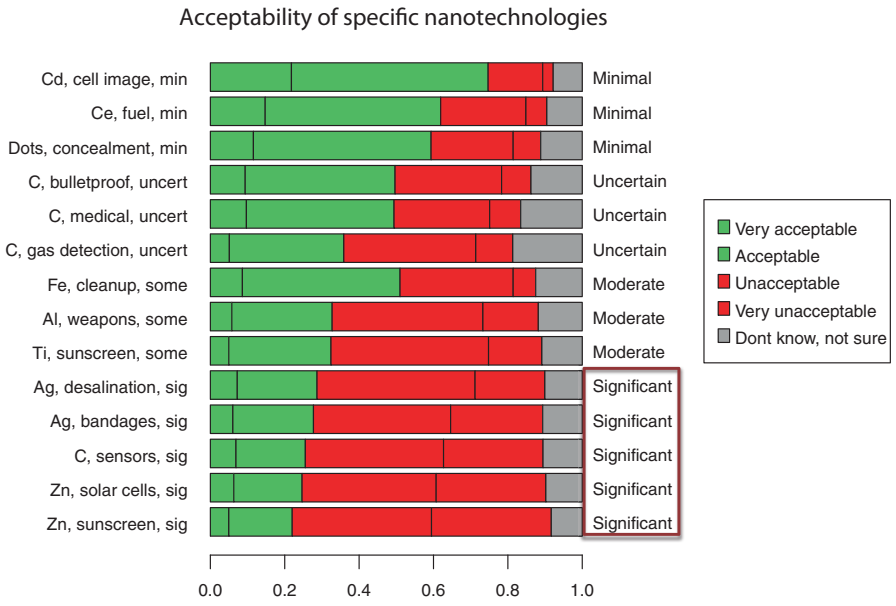


Fig. 3 Risk signal and acceptability of specific nanotechnologies. (Satterfield et al. In preparation (b))

to study participants' own emergent perceptions based on their own relatively naturalistic information seeking, uptake and group dialogue. This is even more important in a context where we wish to assess the effects of deliberative thinking and dialogue on the evolving views of the new technologies. Our approach has been well validated in past research and from other sources (Pidgeon et al. 2013, 2014, 2017).

*Overall, in the upstream situation (low public awareness and knowledge and high scientific uncertainty about risk) that has typified nanotechnologies in the US, UK and elsewhere, the diverse lay public demonstrate high sensitivity and potential malleability in response to information context, be it about benefit, risk, or safety***.**

Equity and Politics Matter

Our work has also extensively explored in quantitative and qualitative work how gender, race and ethnicity, and other aspects of identity and social position/social location affect the way people make sense of new technologies and how they behave in deliberative settings. This work builds on prior work on the sociology and politics of gender (Fenstermaker and West 2002), the intersection of social positions and identities (Alcoff 2006; Barvosa 2008; Bauer 2014), and on gender and race/ethnicity as factors in risk perception, particularly the 'white male effect' (Flynn et al. 1994; Davidson and Freudenburg 1996; Finucane et al. 2000).

We have shown that the 'white male effect' has been misconstrued in a large proportion of studies using or testing this concept (Satterfield, Findlater & Harthorn Under review), by which we mean it is often cited by others as a gender and race effect per se, while our own research demonstrates a situation where apparent gender and race differences in risk perceptions are in reality explained by other, largely socio-political and vulnerability variables. In our nanotech risk perception survey work, gender and race/ethnicity do again predict acceptability, risk and/or benefit judgments (Conti et al. 2011; Satterfield et al. 2012; Collins et al. In preparation, etc.). However, this result is also consistent with the 'white male effect'—that is, it is largely driven by a subset of white men with relatively higher income, education, and more conservative views who are less concerned with technological risks, and/or by a set of nonwhite women whose social-economic status and political world views are in opposition to this.

Our deliberative work has also closely examined gender (and race/ethnicity) effects. Our US-UK comparative nano energy and nano health and enhancement sessions found strong associated gender effects in the nano health and enhancement, but not the nano energy sessions, and, as we reported, 'social risks' were far more evident than technological risk concerns. These focused on distributive and procedural justice issues by participants who were women and people of color in both countries (Pidgeon et al. 2009). Based on this finding, the following set of US nanotech deliberative workshops was designed to explore gender effects more closely, with a two application conditions (energy, health) by three group composition (women only, men only, mixed women and men) design:

This project has generated a number of papers exploring the 'white male effect' and views on food risk (Rogers-Brown et al. 2011), health and enhancement (Harthorn 2016), technological ambivalence and linked patterns of gender- and race-skewed results (Harthorn et al. 2011b). In one paper (Shearer et al. 2014), we also contextualized participants with 'low risk' views to show that they actually had 'high risk' views when focused on economic risk versus much lower risk views when focused on environmental or health risks. We have studied the highly gendered talk in deliberations—men speak more than women and use more intrusive interruptions; whites use more intrusive interruptions than people of color; women speak more, use more backchannels/cooperative overlaps, and use more self-disclosure when discussing health and human enhancement applications versus energy/environment applications, whereas men's patterns of talk do not vary across applications (Denes et al. In preparation). Thus, subtle and overt group dynamics play a major role in deliberative settings, largely unexamined before this work. Our work demonstrates that privilege and inequality are often implicated in the social risks people attribute to new technologies (Harthorn et al. 2009, Harthorn et al. In preparation).

Public views on new technologies thus clearly reflect issues of identity and power, past experiences, and cultural understandings and preferences. Our work strongly argues for the importance of including such factors in research on public attitudes and perceptions.

Our work has also developed theories of trust in risk research. *Trust* is a critical dimension of diverse public risk perception, and our results also confirmed the trust asymmetry principle (cf. Slovic 1993) for the nanotechnology case—that it is much easier to lose trust than to regain it. In our phone survey based on a representative US sample, we extended this work using *realistic examples* of nanotech applications (designed in collaboration with S&E colleagues) (Satterfield et al. 2012) and discovered:

- A counter finding—that mobility of trust is greatest for those with **positive** predispositions to nano—these respondents demonstrated a greater increase in trust when faced with proactive risk management actions. We see this as indicating an unusual opportunity for dialogue (and part of the benefit perception research above).
- This same survey did find more mobility of views when bad news about risks ***follows*** good than the other way around, showing experimentally the socially risky aspect of benefit only risk communication.
- It also found that affective ambivalence played a greater role in response to positive and negative news stories than for those who report stronger good or bad feelings (Fig. 4).

Lack of trust, in governments and in corporations, is a recurring theme in all our deliberative research. For example, our US-UK comparative nanotech deliberations found lack of trust clearly associated with risk concerns, and more nuanced cross-national differences with UK participants less trusting of government and US participants more skeptical about the trustworthiness of corporations/business. Lack of

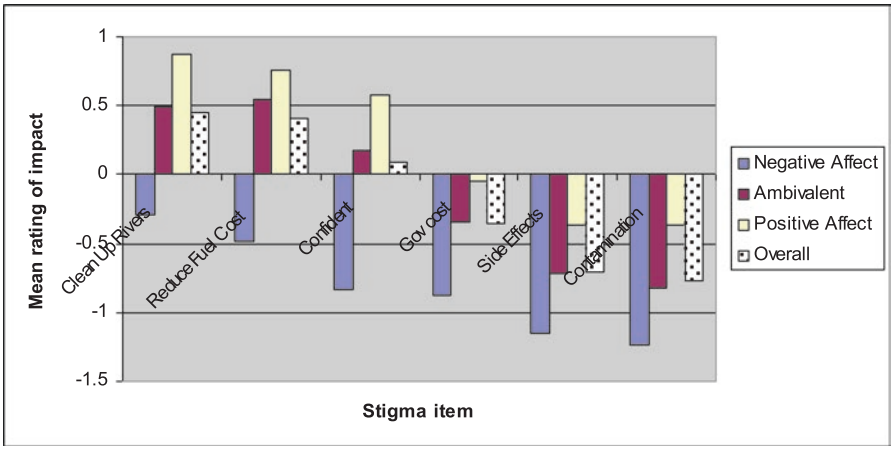


Fig. 4 Mean ratings of six nanotechnology stigma condition statements. Mean ratings are shown for affectively positive, negative and ambivalent participants, as well as 'overall' scores (all participants combined). 'Positive' items are on left of Figure, 'negative' items are on right of Figure. (Corner et al. 2010)

trust also obviously intersects with social and political inequality in ways that all responsible governance needs to take account of—those who have experienced environmental and health harms in the past express the greatest likelihood of future harm and vulnerability, and these views are far more widespread among women and people of color (Conti et al. 2011).

Counter-Intuitive Toxicology

In collaboration with the UC Center for Environmental Implications of Nanotechnology (UC-CEIN), Harthorn, Freudenburg, Kandlikar, and Satterfield, with our students and postdoctoral researchers, have pursued a series of studies with more specific attention to the environmental health and safety, environmental values, and intuitive toxicology aspects of ENMs. We have asked in particular if and how nanotechnologies are unique compared with what is known about other technologies, particularly in reference to perceived environmental risks. We hypothesized that since nanotechnologies have many features common to other technologies perceived as high risk, people may have amplified concerns about them if they know about these characteristics. Among these intuitive factors are: invisibility of risk objects, uncontrollability, scientific uncertainty, ubiquity, perceived toxicity, and risks to future generations. However, intuitive judgments about risk are argued to derive from rapid, "fast-thinking" assessments, heavily informed by affect or emotion in conditions of low knowledge and awareness. We thus also hypothesized that some reliance on one's own sensory apparatus would be at play when 'sensing' hazard, avoiding exposure or assuming that the more material we're exposed to, the

greater the hazard. Such intuitive toxicological assumptions may not help us, for example, if nanomaterials cannot be detected through our senses. For these reasons, we explored multiple dimensions of "intuition" and found (Satterfield et al. 2018; Satterfield et al. In preparation):

1. Rapid intuitions about different environmental media (e.g., air, water, and soil or those situated within biomes, for example mountain-air, -water or -soil) can be captured and predictive of risk attitudes. Four factors underlying intuitive assessments of environmental media were found, with resilience and tangibility emerging as key.
2. Rapid assessments of the perceived resilience of environmental contexts (e.g., recovers easily from harm) were particularly powerful in predicting the acceptability of nanomaterials.
3. Environmental values also correlate with ideas about resilience and environmental justice, but remain discrete constructs when examined via factor analyses or PCAs (principal components analysis).
4. An index of perceived bodily resilience was also developed as part of the perceived risk survey work, and is predictive of the acceptability of different nanomaterials.

Nano Poses a Major Regulatory Challenge

Nanotechnologies have posed numerous challenges to governance and regulation. Our research has contributed a significant body of work in this important area**. In spite of their arguable importance in the upstream nanotech research context, there have been surprisingly few systematic and longitudinal programs of research on nanotechnology experts outside that reported here.**

Regulatory Anxiety

CNS has conducted leading research on the regulatory capacity of the US government to safely handle the challenges posed by nanomaterials. Beaudrie, Kandlikar, Satterfield and Harthorn began this work through analysis of the regulatory process across the full product life cycle (Beaudrie 2010), which identified significant regulatory gaps across the life cycle and across regulating agencies (Beaudrie et al. 2013a). For example, dedicated downstream waste management for engineered nanomaterials is lacking, even though it may be appropriate for some materials. As well, the regulatory presupposition that dose and so bulk load of materials 'makes the poison' may also not apply to the nanoscale. For these and kindred reasons, a large-sample survey of nanoscale experts followed, engaging nano-scientists and engineers, nano-environmental health and safety scientists, and regulatory scientists and decision-makers. This work clearly demonstrates that among these three groups

of experts, those who know the most about the regulatory process—regulators—have the least trust in its preparedness and capacity to handle the challenges of safely and responsibly regulating engineered nanomaterials across the life cycle (Beaudrie et al. 2013b; Beaudrie 2013).

Expert Diversity

This same survey demonstrated the diversity of views across and within expert groups. Across the groups, benefits are perceived to outweigh the risks generally, but notable group differences are evident in Fig. 5. Across 14 different nano-applications (Fig. 6) eight differences in perceived risk were found. A general pattern of difference also reflected where experts were positioned in the nano life cycle. Specifically, natural scientists and engineers working on nano-materials and processes tended to see the risks of nanomaterials as comparatively low, nano environmental health and safety (EHS) scientists see risks as somewhat higher, whereas nano regulators are most inclined to evaluate the risks as comparatively highest. This between group variation is explained in part by perceived novelty of nanomaterials, the perceived uncertainty of the effects of materials, and by those who prefer precautionary versus market-based approaches to governance of these risks (Beaudrie et al. 2014).

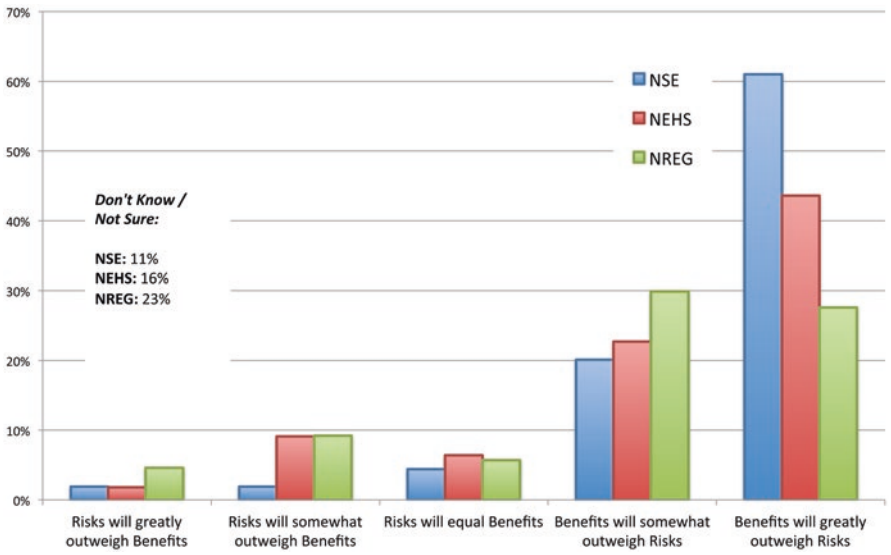


Fig. 5 "Risk versus Benefit" ratings for nanotechnologies in general. Color-coded bars indicate the proportion of respondents in each expert group (NSE, NEHS, and NREG) choosing the indicated response. (Source: Beaudrie et al. *PLOs One* 2014 9(9) e106365, 5 doi:https://doi.org/10.1371/journal.pone.0106365.g001)

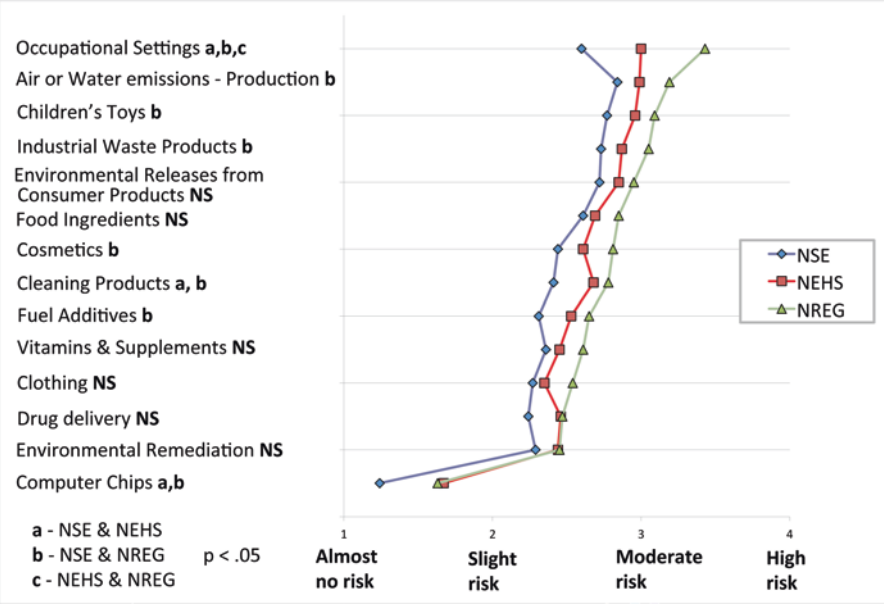


Fig. 6 'Risk Perception' ratings for NSE, NEHS, and NREG expert groups. Mean scores for each group are indicated with points on respective color-coded lines capturing 14 different nanotechnology scenarios rated between 'almost no risk' and 'high risk'. Significant differences in means were determined using a one-way ANOVA with post hoc analysis, and are indicated with a, b, and c markings as outlined in the legend. (Source: Beaudrie et al. 2014 PLOS One 9(9), 3,106365, 6)

In addition, within group differences are evident in that significant gender differences were found in risk ratings for 12 of the applications, with men seeing lower risk and women higher (see Fig. 7). Expert differences could affect safety practices and research decisions as well, and experts are less cohesive than they (and we) think.

Importantly, this work provides evidence that we all, including experts, display "motivated cognition" about risks that is affected by our social positions, values, and a host of other factors.

Industry at Sea

Our group has conducted two major surveys of ENM risk perceptions and health and safety practices in the international nanomaterials industry. The first study was funded by ICON (the International Council on Nanotechnology) in 2006 and led by ecotoxicologist/microbiologist Patricia Holden, in collaboration with Harthorn, Conti, and Appelbaum (Conti et al. 2008). This first study developed a protocol for industry self reporting of an array of EHS program characteristics such as PPE, exposure monitoring, engineering controls, waste disposal product stewardship and risk beliefs. We found uneven practices and distribution of nano-specific EHS

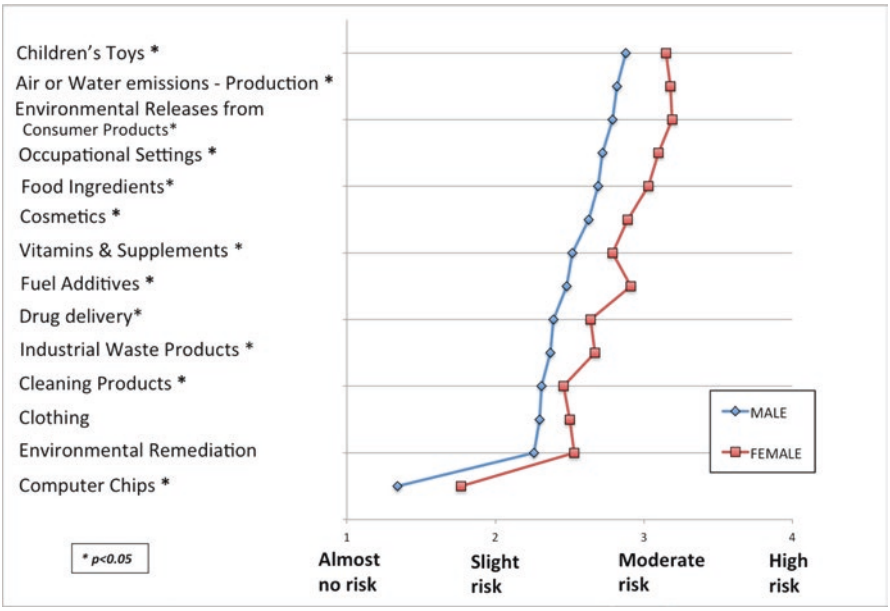


Fig. 7 Experts' risk perceptions differ by gender. (Beaudrie 2013)

practices and expressed need for more guidance on toxicology, exposure and EHS. We (Holden, Harthorn, and Engeman) implemented a second survey in 2009–2010 to reach a broader international sample, assess the impacts of proliferating nano EHS guidance documents around the world, and probe risk perception and attitudes toward regulation in more detail. We found a surprisingly high degree of risk uncertainty across all ENM types, or moderate to high perceived risk—on average almost three quarters (72.5%) indicated uncertainty or moderate-high risk, without much variance across materials. However, although this kind of uncertainty and high risk translates to greater precaution in other groups, with industry we saw a high degree of preference for autonomy from governmental regulation and a low perceived need for self/other protective action—what we might call a risk management stalemate. A majority (59%) agreed that employees are ultimately responsible for their own safety at work. We also found industry attitudes toward the lay public to be negative, reducing prospects for responsible engagement (Engeman et al. 2012; Engeman et al. 2013).

Expert Engagement

UBC collaborator Kandlikar published an influential piece pointing to the impossibility of conducting ENM scientific risk assessment using the business as usual, one material at a time approach (Jae-Young et al. 2009) and several others addressing the importance of alternative methods for expert risk assessment to make risk

analysis and assessment progress in the context of scientific uncertainty and regulatory gaps (Kandlikar et al. 2007; Beaudrie and Kandlikar 2011; Beaudrie et al. 2011). This work provided a careful analysis of the risk information needed and research gaps in need of attention as concerns regulatory decision making. They followed this work by conducting a state-of-the-art Structured Decision Making (SDM) expert workshop tailored to the conditions of high complexity and uncertainty, analytic difficulty and high stakes consequences (Beaudrie et al. 2014), generating new tools for use in this and parallel emerging technology contexts (Beaudrie et al. 2015). *The work demonstrates the importance and feasibility of developing new, methodologically-sound approaches for expert decision making in what can only be called situations of 'regulatory limbo' as is still the case for many nanomaterials.*

Governance and Public Participation: The Art and Science of Public Engagement

Nanotechnologies in the US have been developed within an official rubric of **responsible development**, which *"… implies a commitment to develop and use technology to help meet the most pressing human and societal needs, while making every reasonable effort to anticipate and mitigate adverse implications or unintended consequences"* (National Research Council enter 2006). This ethical vision is articulated in Risk versus Benefit terms: on the risk side of the equation are environmental and human health risks and hazards, as well as wider social risks and disruption; on the benefit side, technologies that answer needs and contribute good to society (Fig. 8).

Although this sounds simple, in practice it's very difficult—how should we weight these different aspects? Whose judgments about both benefits and risks should this be based on? And what processes need to be in place to do the kind of risk analysis and management that incorporates such views? Expert judgment alone is not enough. Democratic public participation is articulated as a key part of responsible development for normative (ethical), instrumental (produce better outcomes), and substantive (incorporate useful information) reasons (Fiorino 1990). So public engagement and participation have been essential elements of the nanotechnology societal implications enterprise at CNS-UCSB (Roco et al. 2011; Harthorn and Mohr 2012a).

Over the course of the life of the CNS at UCSB, a European model for Responsible Research & Innovation (RRI) has crystallized. It more explicitly advocates for technological governance that is: anticipatory, reflexive, inclusive/participatory, and responsive (Owen et al. 2013). This language is not yet widespread in US technological governance terms, but we have compelling evidence that the diverse US public strongly share these ethical stances (Harthorn et al. In preparation). And, as pointed out in our edited volume, "Novel upstream research and engagement efforts

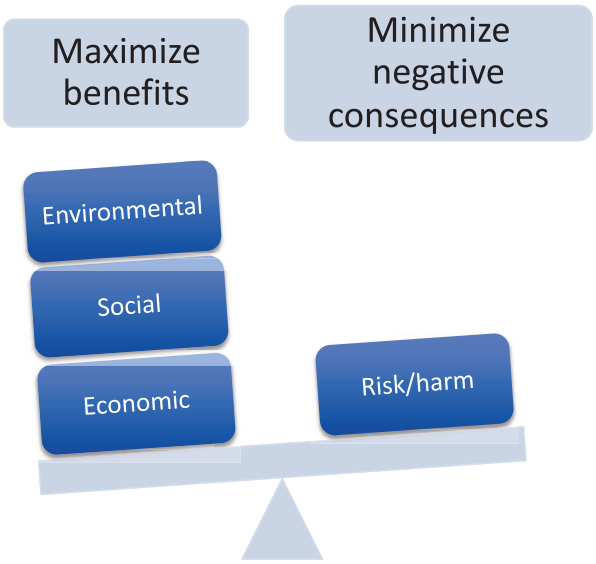


Fig. 8 NAS vision for responsible development of nanotechnologies. (Harthorn 2013)

challenge publics and experts to anticipate feelings, judgments, and actions for whole new classes of technologies, and to imagine them as active agents in social contexts that may reproduce, exacerbate, or ameliorate current inequalities, recreancy [governance failure or incompetence] concerns (Freudenburg 1993), or obstacles to democratic institutions" (Harthorn and Mohr 2012b: 11).

The Public Is Readily Engaged

Even far upstream, meaningful discussion has not only been possible but highly productive in the nanotechnologies case. Our research has contributed at a very prominent level to the development of conceptual thinking about the novel processes of upstream engagement associated with the nanotechnology case. Upstream engagement invites selected members of the public to participate in dialogue about technologies before they are widely researched or known (Wilsdon and Willis 2004; Pidgeon and Rogers-Hayden 2007; Rogers-Hayden et al. 2007; Rogers-Hayden and Pidgeon 2008). Corner and Pidgeon conducted a systematic comparative review of 18 nanotechnology deliberation projects in N. America and Europe and found that the ones that 'worked' shared the following characteristics (Corner and Pidgeon 2012):

- They produced informed judgment, rather than intuitive, 'fast' thinking among participants, that is, actual deliberation took place;
- Benefit centricity was quite widespread;

- But also, they found *latent ambivalence* on the part of the public that was unaffected by increased knowledge and awareness and had as key elements: skepticism toward government & industry; concern about who represents the public's interests; and significant questions about the need for the new technology/product at all.

In the latter case, Corner and Pidgeon (Corner and Pidgeon 2012; also Macnaghten 2010) argue that latent ambivalence "is really about the social context in which a science is conducted, rather than the risks of the technology itself" (and we found this in our own comparative US-UK deliberations). In the latter, concern focused on the social rather than technical side of risk, no matter how much technical info or expertise we provided. We have argued that these new upstream models for successful nanotech upstream engagement can serve an important function in "broadening the scope of public involvement in decision making about science and technologies" (Harthorn and Mohr 2012b: 11).

Our nanotechnology public engagement protocols and success have also served as the foundation for a series of highly successful public engagements in the UK to dialogue on such controversial new technologies as climate geoengineering (Pidgeon et al. 2012, 2013; Corner et al. 2013; Parkhill et al. 2013b) and in the US and UK to discuss shale gas and oil extraction (Thomas et al. 2017; Partridge et al. 2017, 2018; 2019). They have also served as the model for extensive public engagement work in the UK on public values and acceptability of energy system change (Parkhill et al. 2013a; Pidgeon et al. 2014; Demski et al. 2015; Thomas et al. 2018) and on climate change (Corner et al. 2014). *These studies demonstrate the importance of this foundational research on public engagement and citizen dialogue about nanotechnologies' risks and benefits.*

Engaging Organized Groups of the Public

CSOs (Civil Society Organizations—a broader term than non-governmental organization) or SMOs (Social Movement Organizations)-- constitute an important type of public often overlooked in calls for "upstream engagement" which invites participation from individual members of the public in dialogue about technologies before they are widely researched or known (see Corner and Pidgeon 2012; Pidgeon and Rogers-Hayden 2007). Researchers have argued that public engagement projects, rather than creating spaces for public partnership in shaping technological development, may serve as exercises in earning public trust in science experts (Bierle and Cayford 2002). SMOs, however, deliberate nanotechnology in less controlled contexts, through the web, with the media, and within their communication networks (Chilvers and Kearns 2016). As "the uninvited public" (Wynne 2007), SMOs are participating in as well as facilitating upstream engagement, and they are well-positioned to influence public perceptions, particularly in the context of low public awareness of nanotechnology. In comparison to unorganized members of the public, CSOs have better structural and financial resources to conduct research, issue

reports, and communicate their views to the media, policymakers, and industry. Additionally, some CSOs could be understood to represent wider groups of the public in dialogues with government and industry leaders (Engeman, Rogers-Brown & Harthorn 2016; Engeman et al. 2017; Han et al. 2015)

To investigate CSO involvement in nanotechnology development, we (Engemen, Harthorn, Rogers-Brown & Earl) have studied their actions since 2010, building a global database of 233 organizations that have expressed interest in or concern about nanotechnology in English, 101 of whom we identified as 'nano-engaged'—doing more than just endorsing other groups' nano-focused actions. Preliminary findings demonstrate that nano-engaged CSOs targeted government institutions in their pursuit of increased EHS funding, product labeling, and government oversight. Some researchers have argued that, in regard to emerging technologies, CSOs are filling the void left by governments in the wake of neoliberalism (Hess et al. 2008). Despite a seeming lack of trust in government agencies to safeguard consumer and environmental safety, the nano-engaged CSOs in this study, in seeking desired outcomes, targeted government agencies and policymakers, rather than targeting industries directly as might be anticipated based on research in science and technology studies (see Hess et al. 2008).

Public Participation

Our work shows that *public ideas and values about responsible development* include four main factors: (1) the role of the public in tech development, (2) their views on equity and power, (3) their belief in the need for informed consent to move forward with development, and (4) their levels of trust in institutions in the context of nanotechnology. We take this to show that diverse members of the public do have a well-defined and somewhat overlapping set of understandings about responsible development, and that those who feel development is not happening responsibly in these terms are less likely to find environmental exposures of MNM acceptable (Harthorn et al. In preparation) (Fig. 9).

Our work on equity and politics as key drivers shows that gender is just one of many factors that can drive perception and interaction; and we found that group interactions (including multi-stakeholder ones) are socially very complex and difficult to decipher. More work on this and tools for such analysis are badly needed.

Because of our focus on risk concerns, this research has been of significant interest to people in science and engineering (S&E), to the nanomaterials industry, to policymakers and regulators, to NGOs working on related issues, as well as to members of the wider public in the US, Canada, and the UK and EU where the three co-authors are based. We have engaged with state, national and international governing bodies and agencies, S&E audiences, toxicologists and industrial hygienists, the nanomaterials industry, and local and regional communities, science museums, schools, colleges, community colleges, business groups, and civil society groups, extending the work beyond the typical academic venues of disciplinary conferences

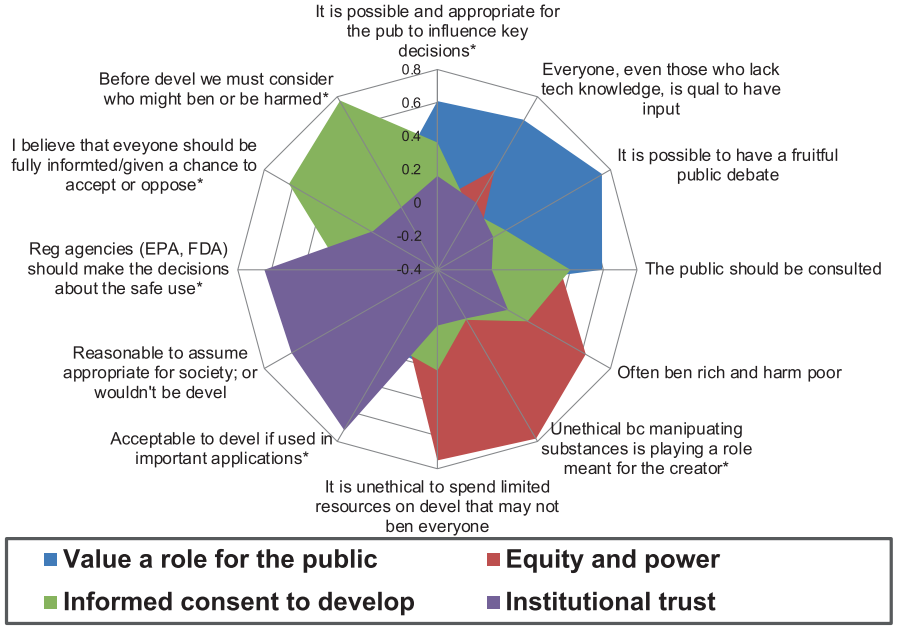


Fig. 9 Public has distinct views on upstream ethics of nanotechnology innovation. Based on web survey of US public n = 697. (Harthorn et al. In preparation. Similar version published in Satterfield et al. 2018 *Ecology & Society*)

and journal publications. Our work has also been covered in the national presses of US, UK, and Canada.

Together, this body of research and our extensive public and expert engagement activities in conjunction with it form an unprecedented effort to link social science research with societal implications of a new set of potentially disruptive technologies.

Summary

- Public acceptability of nanotechnologies is driven by: benefit perception, the type of application, and the risk messages transmitted from trusted sources and their stability over time; therefore transparent and responsible risk communication is a critical aspect of acceptability.
- Social risks, particularly issues of equity and politics, are primary, not secondary, drivers of perception and need to be fully addressed in any new technology development, not just nanotechnologies. We have devoted particular attention to studying how gender and race/ethnicity affect risk judgments.
- The upstream dominance of benefit perception should not be taken as an indication of continued high public acceptability of nanotechnologies over time. Conclusions regarding current views are tempered by a high level of uncertainty and indicate the above noted malleability, particularly if benefit-only communication is followed by risk communication. Public acceptability should be viewed as conditional, requiring continued trustworthy actions by government and industry.
- There is almost no sensitivity among public groups to differences in the actual engineered nanomaterials, even though toxicological evidence indicates increasingly solid evidence for their differential effects. Therefore, the whole class of nanomaterials is vulnerable in the event that more hazardous materials are not regulated well and so become the basis for stigma or radiating effects, similar to chemicals.
- Although representatives from the nanomaterials industry demonstrate relatively high perceived risk regarding engineered nanomaterials, they likewise demonstrate low sensitivity to variance in risks across type of engineered nanomaterials, and a strong disinclination to regulation. This situation puts workers at significant risk and requires regulatory action now (above and beyond the currently favored voluntary or ‘soft law’ approaches).
- All stakeholders in the nano-enterprise, including experts, display dependence in some circumstances on intuitive risk judgments that are at odds with current evidence. Systematic social science research is therefore a critical part of responsible policy and can be used to anticipate where experts most need research and extension support.
- Scientists and engineers, toxicologists, and regulators display significant diversity in their views on the risks of specific nanomaterials and the regulatory suf-

ficiency of current frameworks for regulating this range of nanomaterials and nano-enabled products. Therefore, a diverse composition of experts is needed in regulatory decision-making bodies in order to capture the full range of these views.

- Those scientists and engineers working most closely with nanomaterials in the early stages of development (e.g., of novel materials and applications) show the highest risk tolerance among experts. The implications for labs and bench science safety among students, postdocs and workers should thus be investigated.
- Among experts, nanotech regulators and federal and state agency personnel express the least confidence in the current regulatory system. There are clearly identified gaps (often large) in regulatory coverage across product lifecycles that contribute to these concerns. The aging regulatory system in place demands systematic policy maker attention and integration across agencies.
- In spite of regulatory and risk assessment uncertainties, diverse expert engagement for development of new tools and approaches can be conducted successfully using current theory and practice in structured decision making. It is critical to implement these now rather than to wait for completed hazard and exposure assessments, particularly given this large and complex class of new materials such as engineered nanomaterials.
- The public can and should be engaged, early and often, in the development and commercialization of new technologies, particularly those with high potential for risk (health, environment, and social) and disruption. European deliberative models have been successfully implemented in the US by CNS and could be scaled up for national deliberation. CNS research has shown that a majority of the US public endorse the core values of responsible innovation.
- Civil society organizations such as NGOs can and should be invited participants and have an increasingly important role to play in safe and responsible development and innovation. Societal experts provide important evidence-based knowledge and understanding for effective facilitation of this process.
- Experts can and should be productive and reflexive participants in public engagement. The CNS at UCSB provides hundreds of examples of successful expert engagement, facilitated by social science researchers and based on solid social science evidence. Federal funders should require such integrated efforts and dedicated resources for all new technology R&D.
- Public participation has been greatly enhanced in the NNI through NSF investment in national societal research and education centers. This approach can and should become an integral part of US technology development, with funding and incentives to develop new methods and approaches, grounded in the best social research practices.

Acknowledgements Funding for the research in this chapter was provided by the US National Science Foundation through cooperative agreements #SES 0531184 and #SES 0938099 to the Center for Nanotechnology in Society at UCSB and #SES 0824042 and #SES 1535193 to first author Harthorn. Additional support was provided by NSF and EPA cooperative agreement #DBI 0830117 to the UC CEIN at UCLA. Additional funding was provided by the University of

California at Santa Barbara. Any opinions, findings, and conclusions are those of the authors and do not necessarily reflect the views of the NSF or our other funders.

We would like to thank the anonymous public and expert participants in our research, the constructive critical contributions of a series of panels of NSF external reviewers of the CNS at UCSB, 2006–2016, the steady and often inspiring guidance of our primary NSF program officers and directors: Rochelle Hollander, Stephen Zehr, Michael Gorman, and Frederick Kronz, as well as Mihail Roco, without whom this research would very likely not have taken place. And the staff of the CNS at UCSB whose dedication and support have made this work possible, particularly Bonnie Molitor, Shawn Barcelona, Valerie Kuan, Brandon Fastman, Barbara Gilkes, and many others.

Our main faculty collaborators in this work are listed in full on the website at cns.ucsb.edu and have included: Edwina Barvosa, UCSB, Bruce Bimber, UCSB, Jennifer Earl, Univ of Arizona, Sharon Friedman, Lehigh Univ, William Freudenburg, UCSB (deceased), Robin Gregory, Decision Research, OR, Elizabeth Gwinn, UCSB, Patricia Holden, UCSB, Milind Kandlikar, Univ of British Columbia, CA, John Mohr, UCSB, and Paul Slovic, Decision Research, OR.

Students, postdocs and professional researchers we would like particularly to acknowledge include: Christian Beaudrie, Compass Resource Management, Canada; Karl Bryant, SUNY New Paltz, US; Mary Collins, SUNY ESF, US; Joseph Conti, Univ of Wisconsin-Madison, US; Adam Corner, Cardiff Univ., UK; Christina Demski, Cardiff Univ, UK; Brenda Egolf, Lehigh Univ, US; Cassandra Engeman, Stockholm Univ, Sweden; Darrick Evensen, Cardiff Univ., UK; Shannon Hanna, US Food and Drug Admin, US; Ariel Hasell, Univ of Michigan, US; Mikail Johansson, Gothenburg Research Institute, Sweden; Tristan Partridge, UCSB & Universitat Autònoma de Barcelona, Spain; Tee Rogers-Hayden, Univ of E. Anglia, UK; Louise Stevenson, Bowling Green State Univ, US; and Merryn Thomas, Cardiff Univ., UK.

We also thank editors Jamie R. Lead and Shareen Doak for their comments and suggestions for improving this text.

References

Alcoff L. Visible identities: race, gender and the self. New York: Oxford University Press; 2006.

Barvosa E. Wealth of selves: multiple identities, mestiza consciousness, and the subject of politics. College Station: Texas A&M Press; 2008.

Bauer G. Incorporating intersectionalilty theory into population health research methodology: challenges and the potential to advance health equity. Soc Sci Med. 2014;110:10–7.

Beaudrie C. Emerging nanotechnologies and life cycle regulation: an investigation of federal regulatory oversight from nanomaterial production to end of life. Philadelphia: Chemical Heritage Foundation; 2010. p. 1–63.

Beaudrie C. From cradle-to-grave at the nanoscale: expert risk perceptions, decision-analysis, and life cycle regulation for emerging nanotechnologies. Doctoral thesis, University of British Columbia; 2013 . Available for download at: https://open.library.ubc.ca/cIRcle/collections/ubctheses/24/items/1.0073597

Beaudrie C, Kandlikar M. Horses for courses: risk information and decision making in the regulation of nanomaterials. J Nanopart Res Spec Focus Govern Nanobiotechnol. 2011;13(4):1477–88.

Beaudrie C, Kandlikar M, Ramachandran G. Using expert judgment for risk assessment. In: Ramachandran G, editor. Assessing nanoparticle risks to human health. Maryland Heights: Elsevier; 2011. p. 109–38.

Beaudrie C, Kandlikar M, Satterfield T. From cradle-to-grave at the nanoscale: gaps in US regulatory oversight along the nanomaterial life cycle. Environ Sci Technol. 2013a;47(11):5524–34.

Beaudrie C, Satterfield T, Kandlikar M, Harthorn BH. Expert views on regulatory preparedness for managing the risks of nanotechnologies. PLoS One. 2013b:e80250.

Beaudrie C, Satterfield T, Kandlikar M, Harthorn BH. Scientists versus regulators: precaution, novelty & regulatory oversight as predictors of perceived risks of engineered nanomaterials. PLoS One. 2014;9(9):e106365.
Beaudrie CH, Kandlikar M, Gregory R, Long G, Wilson T. Nanomaterial risk screening: a structured approach to aid decision making under uncertainty. Environ Syst Decis. 2015;35(1):88–109.
Bickerstaff K, Simmons P, Pidgeon NF. Constructing responsibility for risk(s): negotiating citizen-state relationships. Environ Plan A. 2008;40:1312–30.
Bierle TC, Cayford J. Democracy in practice: public participation in environmental decisions. Washington, DC: Resources for the Future; 2002.
Bush J, Moffatt S, Dunn C. "Even the birds round here cough": stigma, air pollution and health in Teeside. Health Place. 2001;7:47–56.
Butler C, Parkhill KA, Pidgeon NF. Nuclear power after Japan: the social dimensions. Environ Sci Policy Sustain Dev. 2011;53(6):3–14.
Casson R. Schemata in cognitive anthropology. Annu Rev Anthropol. 1983;12:429–62.
Chilvers J, Kearns M, editors. Remaking participation. London: Routledge; 2016.
Collins M, Copeland L, Harthorn BH, Satterfield T. Rating the risks: The non-white female effect; In preparation
Conti J, Killpack K, Gerritzen G, Huang L, Mircheva M, Delmas M, Appelbaum R, Harthorn BH, Holden P. Health and safety practices in the nanotechnology workplace: results from an international survey. Environ Sci Technol. 2008;42(9):3155–262.
Conti J, Satterfield T, Harthorn BH. Vulnerability and social justice as factors in emergent US nanotechnology risk perceptions. Risk Anal. 2011;31(11):1734–48.
Corner A, Pidgeon N. Nanotechnologies and upstream public engagement: dilemmas, debates and prospects? In: Harthorn BH, Mohr J, editors. The social life of nanotechnology. New York: Routledge; 2012. p. 247–83.
Corner A, Satterfield T, Pidgeon N, Harthorn BH. Affective ambivalence & nanotechnologies. Presentation at the annual meetings of the society for risk analysis, 8 December. Salt Lake City, Utah, US; 2010.
Corner A, Parkhill K, Vaughan N. Messing with nature: exploring public perceptions of geoengineering in the UK. Glob Environ Chang. 2013;23(5):938–47.
Corner A, Markowitz E, Pidgeon N. Public engagement with climate change: the role of human values. Wiley Interdiscip Rev Clim Chang. 2014;5(3):411–22.
Davidson D, Freudenburg WR. Gender and environmental risk concerns: a review and analysis of available research. Environ Behav. 1996;28:302–39.
Demski C. Public perceptions of renewable energy technologies: Challenging the notion of widespread support. Doctoral thesis, Cardiff University; 2011
Demski C, Butler C, Parkhill K, Spence A, Pidgeon N. Public values for energy system change. Glob Environ Chang. 2015;34:59–69.
Denes A, Cranfill R, Whirlow J, Hanna S, Shearer C, Rogers-Brown J, Harthorn BH. Gender, talk and group dynamics in nanotechnology public deliberation; In preparation.
Devine-Wright P. Reconsidering public attitudes and public acceptance of renewable energy technologies: a critical review. ESRC Working paper 1.4, Research Councils Energy Programme, School of Environment and development, University of Manchester, UK; 2007. Available on line at: http://geography.exeter.ac.uk/beyond_nimbyism/deliverables/bn_wp1_4.pdf
Dietz T, Stern P, editors. Public participation in environmental assessment and decision making. Washington, DC: National Academies Press; 2008.
Engeman C, Harthorn BH. Mobilizing in the context of uncertainty: Social movement organizations and contentious issues of nanotechnology safety, governance and responsible development. Paper presented at the 1st annual environmental politics conference, Bren School of Environmental Science and Management, UCSB, May 31; 2013.
Engeman C, Baumgartner L, Carr B, Fish A, Meyerhofer J, Satterfield T, Holden P, Harthorn BH. Governance implications of nanomaterials companies' inconsistent risk perceptions and safety practices. J Nanopart Res. 2012;14(749):1–12.

Engeman C, Baumgartner L, Carr B, Fish A, Meyerhofer J, Satterfield T, Holden P, Harthorn BH. The hierarchy of environmental, health, and safety practices, in the US nanotechnology workplace. J Occup Environ Hyg. 2013;10(9):487–95.
Engeman C, Rogers-Brown J, Harthorn BH. Mobilizing in the context of uncertainty: social movement organizations and contentious issues of nanotechnology safety, governance, and responsible development. Paper presented at the annual meeting of the Society for the Study of Social Problems, session 161, Montreal, Quebec, Aug 13; 2017.
Erikson K. A new species of trouble: the human experience of modern disasters. New York: W. W. Norton & Co; 1994.
Fenstermaker S, West C. Doing gender, doing difference: inequality, power and institutional change. New York: Routledge; 2002.
Finucane ML, Slovic P, Mertz CK, Flynn J, Satterfield T. Gender, race, and perceived risk: the 'white male' effect. Health Risk Soc. 2000;2:159–72.
Fiorino D. Citizen participation and environmental risk: a survey of institutional mechanisms. Sci Technol Hum Values. 1990;15(2):226–43.
Flynn J, Slovic P, Mertz CK. Gender, race, and perception of environmental health risks. Risk Anal. 1994;14:1101–8.
Freudenburg W. Risk and recreancy: weber, the division of labor, and the rationality of risk perceptions. Soc Forces. 1993;71(4):909–32.
Friedman S, Egolf B. A longitudinal study of newspaper and wire service coverage of nanotechnology risks. Risk Anal. 2011;31(11):1701–17.
Friedman S, Egolf B. Perspective: what have the mass media been reporting on nanotechnology risks? In: Priest SH, editor. Nanotechnology and the public: risk perception and risk communication. Boca Raton: CRC Press; 2012. p. 157–65.
Gregory R, Satterfield T, Hasell A. Using decision pathway surveys to inform climate engineering policy choices. Proc Natl Acad Sci. 2016;113(3):560–5.
Grove-White R, Macnaghten P, Mayer S, Wynne B. Uncertain world: GMOs, food and public attitudes in Britain. Lancaster: CSEC and Unilever; 1997.
Guston DH, Sarewitz D. Real-time technology assessment. Technol Soc. 2002;24:93–109.
Hagendijk R, Irwin A. Public deliberation and governance: engaging with science and technology in contemporary Europe. Minerva. 2006;44(2):167–84.
Han X, Engeman C, Appelbaum R, Harthorn BH. Democratizing technologies: assessing the roles of NGOs in shaping technological futures. Santa Barbara: Center for Nanotechnology in Society, University of California, Santa Barbara; 2015. Available for download at: http://www.cns.ucsb.edu/sites/www.cns.ucsb.edu/files/demtech/Democratizing%20Technologies%20Conference%20Report.pdf
Harthorn BH. Nanotechnology multi-stakeholder risk perception: implications for risk analysis, management, and communication. Invited keynote address (and web broadcast), 2013 NNI Risk 3 Stakeholder Workshop, Office of Science and Technology Policy/National Nanotechnology Coordinating Office, Washington, D.C., September 11, 2013.
Harthorn BH. Techno-benefits and social risks. In: Manderson L, Hardon A, Cartwright E, editors. The Routledge handbook of medical anthropology. London: Routledge; 2016. p. 329–37.
Harthorn BH. Nanotechnology. In: Turner BS, editor. The Wiley Blackwell encyclopedia of social theory. Wiley; 2017a.
Harthorn BH. Chapter 44 – Nanotechnologies in societal context. In: Bhushan B, editor. Springer handbook of nanotechnology. 4th ed. Berlin: Springer; 2017b.
Harthorn BH, Mohr JW, editors. The social life of nanotechnology. New York: Routledge; 2012a.
Harthorn BH, Mohr J. Introduction: the social scientific view of nanotechnologies. In: Harthorn BH, Mohr J, editors. The social life of nanotechnology. New York: Routledge; 2012b. p. 1–15.
Harthorn BH, Bryant K, Rogers J. Gendered risk beliefs about emerging nanotechnologies in the US. Paper presented at the University of Washington Center for Workforce Development, Seattle, WA; 2009. Published online at: http://depts.washington.edu/ntethics/symposium/index.shtml

Harthorn BH, Satterfield T, Pitts A, D'Arcangelis G, DeVries L. Intuitive cognition in the perception of environmental media and nanomaterials: a study of US public views. Presented at the international conference on environmental implications of nanotechnologies, Duke University, May 9; 2011a.

Harthorn BH, Shearer C, Rogers J. Exploring ambivalence: techno-enthusiasm and skepticism in US nanotech deliberations. In: Zuelsdorf T, editor. Quantum engagements: social reflections of nanoscience and emerging technologies. Amsterdam: IOS Press; 2011b. p. 75–89.

Harthorn BH, Rogers J, Shearer C, Martin T. Debating nanoethics: U.S. public perceptions of nanotechnology applications for energy and the environment. In: Scott D, Francis B, editors. Debating science: deliberation, values, and the common good. 2nd ed. New York: Prometheus Books; 2012. p. 227–49.

Harthorn B, Bryant K, Rogers-Brown J, Shearer C. Inequality, risk and difference in deliberations about new technologies; In preparation.

Harthorn B, Collins M, Satterfield T. Upstream ethics and nanotechnologies in the US; In preparation.

Harthorn BH, Halcomb L, Partridge T, Thomas M, Enders C, Pidgeon N. Health risk perception and shale development in the UK and US. Health Risk Soc. 2019;21(1–2):35–56.

Hasell A. Risk in social media: public perceptions of shale gas and oil extraction by hydraulic fracturing in the US and UK. Presented at the 2016 annual conference of the society for applied anthropology in Vancouver, BC, April 2016.

Hasell A, Hodges H What's at risk? A comparison of public discussion of fracking risks in Twitter in the US & UK. Presented at society of risk analysis, Arlington, VA, December 2015.

Hess D, Breyman S, Campbell N, Martin B. Science, technology, and social movements. In: Hackett E, Amsterdamska O, Lynch M, Wajcman J, editors. The handbook of science and technology studies. Cambridge, MA: The MIT Press; 2008. p. 473–98.

Holden P, et al. Considerations of environmentally relevant test conditions for improved evaluation of ecological hazards of engineered nanomaterials. Environ Sci Technol. 2016;50(12):6124–45.

Horlick-Jones T, Walls J, Rowe G, Pidgeon NF, Poortinga W, Murdock G, O'Riordan T. The GM debate: risk, politics and public engagement. London: Routledge; 2007.

Irwin A, Simmons P, Walker G. Faulty environments and risk reasoning: the local understanding of industrial hazards. Environ Plan A. 1999;31:1311–26.

Jae-Young C, Ramachandran G, Kandlikar M. The impact of toxicity testing costs on nanomaterial regulation. Environ Sci Technol. 2009;43(9):3030–4.

Kandlikar M, Ramachandran G, Maynard A, Murdock B, Toscano W. Health risk assessment for nanoparticles: a case for using expert judgment. J Nanopart Res. 2007;9(1):137–56.

Kasperson RE, Kasperson JX. The social amplification and attenuation of risk. Ann Am Acad Pol Soc Sci. 1996;545:95–105.

Kurath M, Gisler P. Informing, involving or engaging: science communication in the ages of atom-bio- and nanotechnology. Public Underst Sci. 2009;21(4):447–64.

Leiserowitz A. Climate change risk perception and policy preferences: the role of affect, imagery, and values. Clim Chang. 2006;77:45–72.

Lively E, Conroy M, Weaver D, Bimber B. News media frame novel technologies in a familiar way: nanotechnology, applications, and progress. In: Harthorn BH, Mohr J, editors. The social life of nanotechnology. New York: Routledge; 2012. p. 223–40.

Macnaghten P. Animals in their nature: a case study of public attitudes on animals, genetic modification and 'nature'. Sociology. 2004;38(3):533–51.

Macnaghten P. Researching technoscientific concerns in the making: narrative structures, public responses, and emerging nanotechnologies. Environ Plan A. 2010;42(1):23–37.

Morgan MG, Fischhoff B, Bostrom A, Atman C. Risk communication: a mental models approach. Cambridge: Cambridge University Press; 2001.

Morton T. Hyperobjects: philosophy and ecology after the end of the world. Minneapolis: University of Minnesota Press; 2013.

National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Nanomaterials and nanotechnology. https://www.nicnas.gov.au/chemical-information/Topics-of-interest2/subjects/nanomaterials-nanotechnology. Accessed 15 Mar 2019.

National Research Council. A matter of size: triennial review of the national nanotechnology initiative. Washington, DC: The National Academies Press. 2006. https://doi.org/10.17226/11752.

National Nanotechnology Initiative (NNI). 2019. https://www.nano.gov/about-nni. Accessed 15 Mar 2019.

OECD. Public attitudes to nuclear power. Nuclear Energy Agency, NEA No. 6859; 2010, p. 1–53. Available for download at: https://www.oecd-nea.org/ndd/reports/2010/nea6859-public-attitudes.pdf

Owen R, Stilgoe J, Macnaghten P, Gorman M, Fisher E, Guston D. A framework for responsible innovation. In: Owen R, Bessant J, Heintz M, editors. Responsible innovation: managing the responsible emergence of science and innovation in society. London: Wiley; 2013. p. 27–50.

Parkhill K, Demski C, Butler C, Spence A, Pidgeon N. Transforming the UK energy system: public values, attitudes and acceptability: synthesis report. London: UKERC; 2013a. p. 1–48.

Parkhill K, Pidgeon N, Corner A, Vaughan N. Deliberation and responsible innovation: a geoengineering case study. In: Owen R, Bessant J, Heintz M, editors. Responsible innovation. London: Wiley; 2013b. p. 219–40.

Partridge T, Thomas M, Harthorn B-H, Pidgeon N, Hasell A, Stevenson L, Enders C. Seeing futures now: emergent US and UK views on shale development, climate change and energy systems. Glob Environ Chang. 2017;42:1–12. https://doi.org/10.1016/j.gloenvcha.2016.11.002.

Partridge T, Thomas M, Pidgeon N, Harthorn BH. Urgency in energy justice: contestation and time in prospective shale extraction in the United States and United Kingdom. Energy Res Soc Sci. 2018;42:138–46.

Partridge, T., Thomas, M., Pidgeon, N, & Harthorn, BH. Disturbed earth: conceptions of the deep underground in shale extraction deliberations in the US and UK. Environ Values. 2019;28(6):641–663

Pidgeon N. Normal accidents. Nature. 2011;477:404–5.

Pidgeon N, Rogers-Hayden T. Opening up nanotechnology dialogue with the publics: risk communication or 'upstream engagement'? Health Risk Soc Special Issue. 2007;9(2):191–210.

Pidgeon N, Kasperson R, Slovic P, editors. The social amplification of risk. Cambridge: Cambridge University Press; 2003.

Pidgeon N, Harthorn BH, Bryant K, Rogers-Hayden T. Deliberating the risks of nanotechnologies for energy and health applications in the United States and United Kingdom. Nat Nanotechnol. 2009;4(2):95–8.

Pidgeon N, Harthorn BH, Satterfield T. Nanotechnology risk perceptions and communication: emerging technologies, emerging challenges. Risk Anal (Special Issue). 2011a;31(11):1694–700.

Pidgeon N, Harthorn BH, Satterfield T, editors. Nanotechnologies risk perception and communication (special collection). Risk Anal. 2011b;31(11):1694–783.

Pidgeon N, Corner A, Parkhill K, Spence A, Butler C, Poortinga W. Exploring early responses to geoengineering. Phil Trans R Soc A. 2012;307(1974):4176–96.

Pidgeon N, Parkhill K, Corner A, Vaughan N. Deliberating stratospheric aerosols for climate geoengineering and the SPICE project. Nat Clim Chang. 2013;3(5):451–7.

Pidgeon N, Demski C, Butler C, Parkhill K, Spence A. Creating a national citizen engagement process for energy policy. Proc Natl Acad Sci U S A. 2014;111(Suppl 4):13606–13.

Pidgeon N, Harthorn B, Satterfield T, Demski C. Cross-national comparative communication about the risks of nanotechnologies. In: Jamieson KH, Scheufele D, Kahan D, editors. Oxford handbook on the science of science communication. Oxford: Oxford University Press; 2017. p. 141–56.

REACH (European Commission Registration, Evaluation, Authorisation and Restriction of Chemicals. 2019. http://ec.europa.eu/environment/chemicals/nanotech/index_en.htm. Accessed 15 Mar 2019.

Rip A, Misa T, Schot JW, editors. Managing technology in society: the approach of constructive technology assessment. London: Pinter Publishers; 1995.
Roco M, Harthorn BH, Guston D, Shapira P. Innovative and responsible governance of nanotechnology for societal development. J Nanopart Res. 2011;13(9):3557–90.
Rogers J, Shearer C, Harthorn BH, Martin T. Different uses, different responses: exploring emergent cultural values through public deliberation. In: Harthorn BH, Mohr J, editors. The social life of nanotechnology. New York: Routledge; 2012. p. 195–222.
Rogers-Brown J, Shearer C, Harthorn BH. From biotech to nanotech: public debates about technological modification of food. Environ Soc Adv Res. 2011;2(1):149–69.
Rogers-Hayden T, Pidgeon N. Moving engagement "upstream"? Nanotechnologies and the Royal Society and Royal Academy of Engineering's inquiry. Public Underst Sci. 2008;16:345–64.
Rogers-Hayden T, Mohr A, Pidgeon N. Introduction: engaging with nanotechnologies – engaging differently? Nanoethics Special Issue. 2007;1(2):123–76.
Royal Society & the Royal Academy of Engineering. Nanoscience and nanotechnologies: opportunities and uncertainties. London: Royal Society; 2004. Available for download at: https://royalsociety.org/~/media/Royal_Society_Content/policy/publications/2004/9693.pdf
Satterfield T, Kandlikar M, Beaudrie C, Conti J, Harthorn BH. Anticipating the perceived risk of nanotechnologies: will they be like other controversial technologies? Nat Nanotechnol. 2009;4:752–8.
Satterfield T, Conti J, Harthorn BH, Pidgeon N, Pitts A. Understanding shifting perceptions of nanotechnologies and their implications for policy dialogues about emerging technologies. Sci Public Policy. 2012;40(2):247–60.
Satterfield T, Collins M, Harthorn B. Perceiving resilience: understanding people's intuitions about the qualities of air, water, and soil. Ecol Soc. 2018;23(4):47. https://doi.org/10.5751/ES-10637-230447.
Satterfield T, Collins M, Copeland L, Harthorn B. Bodily resilience as a new measure of intuitive toxicology; In preparation.
Satterfield T, Harthorn BH, Collins M, Pitts A. Resilience and intuitive cognition as predictors of the environmental impacts of engineered nanomaterials; In preparation (a).
Satterfield T, Harthorn BH, Collins M. Comparative acceptability of specific nanotechnologies. In preparation (b).
Satterfield T, Findlater K, Harthorn BH. A quarter century of gender and racial stereotyping in the study of perceived environmental health risks. Under review
Shearer C, Rogers-Brown J, Bryant K, Cranfill R, Harthorn BH. Power and vulnerability: contextualizing "low risk" views of environmental and health hazards. In: Maret S, editor. William R. Freudenburg, A life in social research, Research in social problems and public policy, vol. 21. Bingley: Emerald Group; 2014. p. 235–57.
Slovic P. Perceived risk, trust and democracy. Risk Anal. 1993;13(6):675–82.
Slovic P, editor. The perception of risk. London: Earthscan; 2000.
Slovic P, editor. The feeling of risk. London: Earthscan; 2010.
Stocking G, Hasell A. Twitter as a tool for public engagement with emergent technologies? Top poster presentation at the conference, Democratizing technologies: assessing the roles of NGOs in shaping technological futures conference, University of California, Santa Barbara, November 2014.
Stoetzler M, Yuval-Davis N. Standpoint theory, situated knowledge and the situated imagination. Fem Theory. 2002;3:315–33.
Thomas M, Partridge T, Harthorn B-H, Pidgeon NF. Deliberating the perceived risks, benefits, and societal implications of shale gas and oil extraction by hydraulic fracturing in the US and UK. Nature Energy 2, Published online 10 April, 17054; 2017. https://doi.org/10.1038/nenergy.2017.54.
Thomas G, Pidgeon NF, Roberts E. Ambivalence, naturalness and normality in public perceptions of carbon capture and storage in biomass, fossil energy, and industrial applications in the United Kingdom. Energy Res Soc Sci. 2018;46:1–9.

University of California Center for Environmental Implication of Nanotechnology (UCCEIN). http://www.cein.ucla.edu/new/. Accessed on 15 Mar 2019.
Weaver DA, Lively E, Bimber B. Searching for a frame: media tell the story of technological progress, risk, and regulation in the case of nanotechnology. Sci Commun. 2009;31(2):139–66.
Wilsdon J, Willis R. See through science: why public engagement needs to move upstream. London: Demos; 2004.
Wynne B. Public participation in science and technology: performing and obscuring a political–conceptual category mistake. East Asian Sci Technol Soc Int J. 2007;1:99–110.

EU Regulations and Nanotechnology Innovation

David Carlander and Claire Skentelbery

Contents

Abstract The first EU Regulation that mentioned nanomaterials was published in 2008 (EC) No 1333/2008) on Food Additives), and since then several Regulations have been adopted that requires specific information for nanomaterials that are put on the EU market. In 2011 the European Commission adopted a recommendation for a nanomaterial definition (1–100 nm), that has been implemented e.g. in the Biocidal Product Regulation. The main EU chemical Regulation, REACH, was updated in 2018 with modification of several of its Annexes to require information on nanoforms of chemical substances. Many regulations in the food area have nano-specific provisions as well as in cosmetics and medical devices. Several EU Member States, France, Belgium, Denmark and Sweden require specific registration of nanomaterials that are put on their national markets. Responsible research and innovation and safe(r) by design concepts are being developed to bring nanotechnology products to the market by optimising resources.

Keywords Nanotechnology · Regulation · Legislation · EU · REACH · Nanoform · Nanomaterial · Safe(r) by design

D. Carlander · C. Skentelbery (✉)
Nanotechnology Industries Association aisbl, Brussels, Belgium
e-mail: office@nanotechia.org

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6_8

EU Regulations: An Introduction

Laws within the European Union are based on core principles of human dignity and human rights, freedom, democracy, equality and the rule of law, enshrined in the Treaty of the European Union (TEU) and in the Treaty on the Functioning of the European Union (TFEU) (European Union 2020). The TEU was first signed in 1992 in Maastricht, thus often still referred to as the Maastricht Treaty and the last amendment was agreed in Lisbon in 2007, when the TFEU was also signed. Both were unanimously agreed, at the time, by all the Members States of the EU and they set out the four freedoms which are movement for goods, services, capital and people. The core principles and the four freedoms in the EU are implemented through legislative instruments. There are three main legally binding instruments in the EU; Regulations, Directives and Decisions, as presented in article 288 of the TFEU.

EU Regulations are directly applicable in all EU Member States whereas **Directives** have to be implemented into national laws to become legally binding. Finally, a **Decision** is directed to an individual or a company. An example of a Decision would be where the European Commission would directly intervene to insist that a specific company or an individual perform a specific measure. Such specific measures could include instruction to withdraw a product from the market.

As described in the TEU and TFEU EU law such as Regulations, Directives and Decisions takes precedence over national laws. A Directive can be considered to set out an aim of what should be achieved, whereas the practical route to achieve this aim can be performed differently in Member States. An adopted Directive therefore also sets out deadlines when Member States should have implemented the Directive in its national regulations.

The EU has slowly transitioned to creating more and more legislation in the form of Regulations. The advantage of Regulations are that they are applicable throughout the union and thus allow for a harmonised system, more easily managed by industries as well as by regulators and thus facilitating an understanding of the regulatory framework in EU. Nowadays, the EU approves on average 80 directives, 1200 regulations and 700 decisions per year (Toshkov 2014).

The European Commission, which is the EU Executive body, shape the EU's overall strategy, proposes new EU laws and policies, monitors their implementation and manages the EU budget. The European Commission is the only EU body that has the authority to initiate a legislative process of creating a new Regulation, a Directive or a Decisions. A legislative proposal from the EC can be vetoed or amended during the legislative process by the European Council or by the European Parliament. The European Council is made of governmental representatives of all EU Member States, and the European Parliament is composed of parliamentarians elected every five year in European wide national elections (see Fig. 1 for the EU institutional triangle). The process put in place by the EC to present a legislative proposal follows a process that usually starts with the publication of a White Paper. A White Papers contain proposals for European Union action in a specific area. The purpose of a White Paper is to launch a debate with the public, stakeholders, the European Parliament and the Council in order to arrive at a political consensus. A

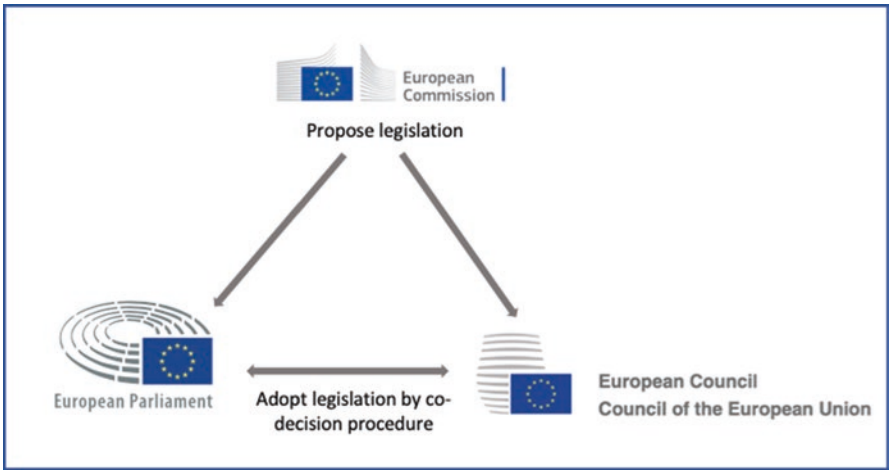


Fig. 1 The EU institutional triangle

White Paper can outline several types of possible issues that the EU should tackle, e.g. with regulatory actions, that could be developed over a longer timeframe, usually 5–10 years. Members States are consulted about the White paper, and comments are invited from the public which includes interested stakeholders such as industries, as well as individual citizens. The White Paper often outlines indicative time frames for when the EC is planning to present a legislative proposal to the European Council and the European Parliament. Before a legislative proposal is presented, a substantive amount of preparatory work takes place, including the EC asking for reports to be drafted by e.g. EU agencies in the sector in question, or by tendering out the drafting to consultants. The EC also has to prepare an impact assessment evaluating different scenarios that could arise depending on various possible regulatory measures.

Once the regulatory proposal is finalised by the EC, it is being officially presented to the European Council (2020) and to the European Parliament (2020), where discussions will then take place in relevant Committees. After amendments and agreement, the proposal, is adopted and published as a new legislative act in the Official Journal of the EU (2020). Once the legislation is published, it usually enters into force 20 days after publication as it is then an official EU regulatory act that must be complied with.

Introduction to the EU REACH Legislation

The main legislation in the EU for chemicals is the REACH Regulation, published in 2006. The acronym REACH stands for Registration, Evaluation, Authorisation and Restriction of Chemicals (EC no 1970/2006) (Juncker 2018). The Regulation is

made up of 141 Articles followed by 17 Annexes and applies to manufacturing and import of chemical substances, mixtures and articles as well as certain substances used research and development purposes. REACH provides a definition of a substance in Article 3 as 'a chemical element and its compounds in the natural state or obtained by any manufacturing process'.

The Regulation was negotiated for many years between the European Commission, the Council and the Parliament and was adopted following the procedure outlined above. REACH places the burden on industry to provide dossiers with information on the substances used on the EU market under the 'no data – no market' notion. The Regulation is managed by the European Chemicals Agency (ECHA) based in Helsinki, Finland (European Commission 2020a).

The REACH Regulation is closely connected to another Regulation from 2008 on Classification, Labelling and Packaging of Substances and Mixtures EC no 1272/20089 abbreviated as CLP (Pöttering and Le Maire 2008). The CLP Regulation classifies substances as hazardous when they meet classification criteria in CLP. Hazards of a substance are identified by assigning a certain hazard class and category. The hazard classes in CLP cover physical, health, environmental and additional hazards. Once a hazard is classified it consequently needs specific labelling and packaging. The CLP provides requirements to apply classification specifications for substances and how hazard labels should be presented on labels and the packaging. The CLP incorporates the Global Harmonised System by the UN into European legislation which harmonises hazard classification of substances.

Figure 2 provides an overview of EU regulations dealing with nanomaterials, in one way or another, where REACH and CLP are horizontal regulations dealing with chemicals in general, and where vertical regulations are dealing with specific areas or sectors.

The requirement to register a substance, mixture or an article under REACH and provide a dossier with information is based on annual tonnage produced in, or imported into, the EU market. The general rule is that all substances with an annual production over 1 tonne (1000 kg) should be registered. The tonnages are based on a potential exposure, where larger production volumes would consequently mean a

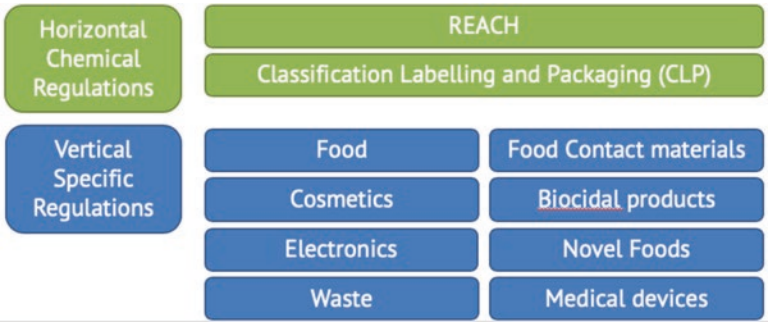


Fig. 2 Overview of EU Regulations dealing with nanomaterials. Each regulation is detailed further in the chapter

larger exposure thus with higher tonnage, more information needs be provided in the dossier. There are 4 tonnage bands; above 1 tonne, 10 tonnes, 100 tonnes and 1000 tonnes. Above 10 tonnes, the dossier should be also accompanied by a chemical safety report (CSR) following Annex I of the Regulation.

The requirement on when to register a substance was staggered over three phases to enable applicants to manage the transition to such a significant new legislative framework. The initial deadline was 2010 for substances over the 1000 tonnes annual production threshold, followed by 2013 for above 100 tonnes and finally May 2018 was the last registration deadline, for substances over 1 tonne.

In addition to substances, REACH also has provisions for mixtures (compositions of two or more substances) as well as articles (i.e. an object which during production is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition).

The information to be provided in a dossier for a substance is provided in the Annexes VII (above 1 tonne) to × (above 1000 tonnes) of the Regulation. Information requirements include information on physico-chemical properties (e.g. melting point, water solubility etc) and toxicological information (e.g. skin irritation/corrosion, acute toxicity etc) and ecotoxicological information (e.g. aquatic toxicity, biodegradability etc). For the higher tonnage bands, more comprehensive information is required, sometimes involving testing on animals.

EU Regulatory History for Nanomaterials

Since publication of the 2004 UK Royal Society of Science (The Royal Society 2004) report, there has been an ongoing discussion among policy makers, including EU Member States, and stakeholders how best to ensure that the specific characteristics of nanomaterials are appropriately covered within EU legislation. This discussion not only focused on how to regulate nanomaterials, but also how do define a nanomaterial.

A Recommended EU Definition of Nanomaterial

Following discussions with stakeholders, policy makers and a public consultation, the European Commission published a Commission Recommendation in October 2011 on the definition of nanomaterial (2011/696/EU) (Potocnik 2011). As it is a recommendation (and not a Regulation), it is not legally binding and has to be transferred into a Regulation (or a Directive) to become legally applicable. However, a recommendation from the European Commission creates a strong message and can be referred to in court. The EC definition is also often referenced in activities outside the EU, possibly due to the comprehensive nature of EU regulatory frameworks

that are often regarded internationally to set the minimum requirements for countries outside EU to trade with EU.

The definition provides guidance how the regulator (i.e. the European Commission) intends to manage the complex issue of how to define nanomaterials in a legally binding manner. The 2011 recommendation states that a 'nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm.' It further states that 'By derogation […] fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nano materials.'

For the purpose of the recommendation, it also clarifies that 'particle' means a minute piece of matter with defined physical boundaries; 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components and 'aggregate' means a particle comprising of strongly bound or fused particles.'

What is interesting to note in the recommendation is that it is solely based on size, as this is a property that can be measured, and thus should the need arise, hold up in court. It is also noteworthy that it also includes natural materials containing particles (Rauscher et al. 2019). The argumentation put forward by the European Commission for this inclusion is that a nanomaterial is based on size, and there could be instances where it could be appropriate to refer and describe natural materials as nanomaterials.

In practice, the accurate measurement of nanomaterial size is often challenging and depends on the size of the nanomaterial to be measured and the method applied, as well as on other aspects (e.g. matrix, elemental composition etc) (Miernicki et al. 2019).

The REACH Regulation and Nanomaterials

The REACH Regulation covers chemical substances (with some agreed exemptions), and consequently also nanomaterials. This was first agreed among EU Members States in 2008 communication from a meeting of CARACAL, a decision-making group of the EU Member States Competent Authorise for REACH (2008) and CLP (European Commission 2020a). However, as industries were registering their substances, the REACH Regulation did not specifically mention nanomaterials, nor place a requirement to provide detailed information on size or other properties. Therefore there was a concern that authorities, including ECHA, would not receive enough information to understand if a substance could have a nanoform, and if the properties of the nanoform would be different from the bulk or soluble form of the registered substance, and if this would impact the safety of the substance.

The European Commission have over the years performed two regulatory reviews on nanomaterials in EU legislation. The first one was performed in 2008 (European

Commission 2008) and the latest one was performed in 2012, where it again was reinforced that the European Commission considers the REACH Regulation to be an appropriate legal act to regulate nanomaterials (European Commission 2012). The second regulatory review stated that '…nanomaterials are similar to normal chemicals/substances in that some may be toxic and some may not. Possible risks are related to specific nanomaterials and specific uses. Therefore, nanomaterials require a risk assessment, which should be performed on a case-by-case basis, using pertinent information. Current risk assessment methods are applicable, even if work on particular aspects of risk assessment is still required.'

The perceived lack of information on nanomaterials in REACH substance dossiers initiated a long, and sometimes heated, discussion between the European Commission, Members States and stakeholders (including industry and civil society organisation) how to best ensure information on nanomaterials are to be provided to authorities. The discussions were initiated after the second regulatory review and continued until the REACH Annexes were adopted in 2018.

After several assessments (European Commission 2019) the European Commission, as the EU policy maker came to the conclusion that nanomaterials are substances and therefore already fall under REACH, that a nanospecific Regulation was not required, and that a modification of the REACH Annexes would be sufficient to request information on nanomaterials.

Amendment of REACH Annexes to Include Nanomaterials and Nanoforms

After many years of internal deliberations and discussions with Member States and stakeholders, the European Commission announced its proposal for a revision of several Annexes of the REACH Regulation in the Autumn of 2017. The proposal, in the form of a European Commission Regulation, was discussed and voted upon in the REACH Committee composed of representatives from the EU Members States. The European Commission then adopted the Regulation and it was published in December 2018 as Commission Regulation EU) 2018/1881 (KEMI 2020). This Regulation is in itself only composed of 3 Articles, but has an Annex setting out changes to 9 (Annex I, III, VI. VII, VIII, IX, X, XI and VII) of the 17 REACH Annexes.

The most profound change is that REACH Annex VI now includes a legally binding definition of nanomaterial, referred to as nanoform in the Annex, that, in turn, is based on the 2011 European Commission recommendation. The term used in Annex VI is not nanomaterial, but nanoform, as a nanomaterial can be considered as another form of a substance. Thus, a substance (as defined in REACH) can have several forms, including nanoforms. Further, an important aspect is that under REACH the concept of 'set of similar nanoforms' can be applied during the registration process. By using sets, a registrant can, for instance, provide a justification

that information from one nanoform can be applied to a wider group of similar nanoforms, thus reducing the amount of information to be provided. How sets will be used in practice will be interesting to find out once applicants are updating, revising or applying for registration under REACH.

Another notable modification of the REACH Annexes is that physicochemical description of nanoforms now has to include information on number-based particle size distribution, specific surface area by volume, morphology and surface functionalisation. The REACH Annexes related to information requirements for tonnages are also modified to include specific considerations, test methods and modifications for when nanomaterials are part of the dossier (Clausen and Hansen 2018).

To facilitate the registration of substances according to the REACH requirements, ECHA has published many guidance documents. As of summer 2019, ECHA is in the process of finalising a guidance document that describes the information to be provided to describe and characterise a nanoform. A draft of the 'Appendix for nanoforms applicable to the Guidance on Registration and substance identification' from June 2019 is available (European Chemicals Agency 2019). ECHA also has specific Annexes to their main guidance document on information requirements and chemical safety testing related to nanomaterials (ECHA 2020).

Vertical Regulations Within the European Union

Although REACH is a Regulation that, in principle, covers almost all chemical substances, there are several sector specific Regulations that can take precedence of REACH. These Regulations are prominent in food, cosmetics, biocides, medical devices, and other areas. The nanospecific aspects of EU sector specific Regulations are discussed below.

Nanomaterials Within Cosmetics

The EU regulates cosmetics in a Regulation from 2009 (EC No 1223/2009 on cosmetic products) (Buzek and Ask 2009) that establishes rules to be complied with by any cosmetic product made available on the EU market. This Regulation was the first EU Regulation that provided a definition of nanomaterials. Article 2(k) defines a 'nanomaterial' as an 'insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm'. This definition is, in many ways, different from the definition used in REACH and in the Biocidal Regulations, as well as the one used in the food area for engineered nanomaterials. The cosmetic definition uses the same size range (1–100 nm) as REACH and in Biocides, but also requires the nanomaterial to be intentionally manufactured and importantly also requires insolubility and biopersistence as its requirements. As a consequence, a substance that is used in several

sectors can be defined as a nanomaterial in one regulation, but not in another regulation. This can of course cause administrative burdens for producers who need to be aware where their product will be used.

To help industries submit a dossier for a nanomaterial to be used in cosmetics, the European Commission Scientific Committee on Consumer Safety (SCCS) published in 2012 a Guidance on the Safety Assessment of Nanomaterials in Cosmetics' (Scientific Committee on Consumer Safety SCCS 2012). This guidance provides information on what an applicant should provide in order for fulfil the requirements in the Regulation and for the SCCS to perform a risk assessment and provide an opinion. As of autumn 2019, the SCCS is in final phases of updating its guidance.

Nanomaterials Within Food Production

Within the EU, there are many Regulations that cover the food sector, ranging from Regulations on plant protection products, genetically modified organisms, hygiene aspects and specific Regulations on e.g. food additives, flavourings, enzymes, novel foods, as well as on food contact materials (i.e. food packaging) and labelling of foods. Many of the food Regulations have been revised to include specifics related to nanomaterials.

In the EU, there is a general food law from 2002 (EC) No 178/2002 (Cox and Piqué i Camps 2002) that, among other things, defines in general terms food as 'any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans.' The general food law also established the European Food Safety Authority (EFSA, based in Italy) to provide scientific opinions on risk assessment issues related to food.

EFSA has published several opinions related to nanomaterials, and in 2018 they published a new 'Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health' (Hardy et al. 2018). The EFSA nanomaterials guidance covers nanospecific aspects and considerations of all types of dossiers that EFSA covers. In this regard, the EFSA guidance focus on aspects that should be considered in addition to the normal guidance document EFSA publishes. E.g. a nanomaterial used as a food contact material should consider the nanospecific guidance in addition to the normal food contact material guidance.

Food Information and Labelling

Labelling of foods and its packaging is regulated in the Food Information to Consumers Regulation from 2011, Regulation (EU) No 1169/2011 on the provision on food information to consumers (Buzek and Dowgielewicz 2011) With respect to nanomaterials this Regulation uses the definition of engineered nanomaterials as found in the Novel Food Regulation (EU) 2015/2283 (Schulz and Schmit 2015). For

the list of ingredients to be used on the food label, the Regulation states that 'all ingredients present in the form of engineered nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word 'nano' in brackets'. Currently, there are no known labelled nano food products on the European market.

Novel Foods

Novel Food is defined as food that had not been consumed to a significant degree by humans in the EU before 15 May 1997, when the first Regulation on novel food came into force. The current Regulation on novel foods (EU 2015/2283) provides a definition of engineered nanomaterials that is different from the 2011 EC recommendation (Schulz and Schmit 2015). Article 3 in this Regulation specifically sets out that food 'consisting of engineered nanomaterials is to be defined as novel food, and this is also applicable to vitamins and minerals if they 'they contain or consist of engineered nanomaterials' (Schulz and Schmit 2015).

The same Article provides a regulatory definition of 'engineered nanomaterial' to mean 'any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale (Schulz and Schmit 2015). Properties that are characteristic of the nanoscale include: (i) those related to the large specific surface area of the materials considered; and/or (ii) specific physico-chemical properties that are different from those of the non-nanoform of the same material.'

As can be noted, the regulatory definition in the novel foods Regulation refers to engineered nanomaterials, and there are several differences compared to the 2011 recommendation as well as to the regulatory definition in the 2018 adopted REACH definition of nanomaterial.

Notably, the novel food definition talks about one or more dimensions 'in the order of 100 nm' which is imprecise compared with the 1–100 nm in REACH. Furthermore, an engineered nanomaterial needs to be intentionally produced, so natural nanomaterials are outside this definition. This is logical, as many constituents within foods are naturally in the nano size range and are thus excluded from the engineered nanomaterial definition.

The novel food definition is unfortunately also imprecise in that it references aspects that are difficult to objectively measure and define, including materials that 'retain properties that are characteristic of the nanoscale'. These characteristics are difficult to describe and agree upon in a regulatory precise manner.

Food Additives

The food additives Regulation from 2008 ((EC) No 1331/2008) (Pottering and Le Maire 2008) is the first EU legislation that specifically mentions nanotechnology, and states in Article 12 concerning changes in the production process of a food additive that '…or there is a change in particle size, for example through the use of nanotechnology…'. This means that in practice that an applicant submitting a dossier for a food additive authorisation needs to provide information on these changes in particle size. The EFSA guidance document for nanomaterials should therefore be followed by food additive applicants.

Food Contact Materials

There is a Regulation from 2004 in EU (EC No 1935/2004 on materials and articles intended to come into contact with food (Borrell Fontelles and Nicolai 2004) that provides an overall framework for all materials intended to come into contact with food. As this Regulation was adopted before the main nano discussions took place, there is no specific mention of nanomaterials or nanotechnology within the text. However, as it is a framework Regulation, it applies to all types of food contact materials, and therefore also nanomaterials are covered under this Regulation, even if they are not specifically mentioned.

In 2011, the Commission published a Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food (Barroso 2011) that specifically includes provisions for nanomaterials. Article 9 states that 'Substances in nanoform shall only be used if explicitly authorised and mentioned in the specifications in Annex I.' Since 2011, several food contact material substances in nanoform have been authorised, based on performed EFSA opinions, to be used in plastic packaging. Such materials include e.g. carbon black, titanium nitride and silicon dioxide. There is no definition of nanomaterials in this Regulation, rather the authorised substances in Annex I have specifications of the substance in question that needs to be fulfilled, e.g. that an authorised material needs to be within a specific size range.

Active and Intelligent Food Contact Materials

The Regulation on active and intelligent food contact materials (EC) No 450/2009 (Vassiliou 2009) apply to food contact materials that have been manufactured to have specific properties to inform about the food, or to release or absorb substances from the food e.g. to increase shelf life. With regard to nanomaterials, as it also an early Regulation, it is rather vague and refers to 'New technologies that engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles, should be

assessed on a case-by-case basis as regards their risk until more information is known about such new technology' (Vassiliou 2009).

Nanomaterials and Biocides

The EU Regulation No 528/2012 concerning the making available on the market and use of biocidal products (Schulz and Wammen 2012) is the first Regulation with a definition based on the 2011 recommendation. However, as a biocide always has an intent, the word 'natural' is removed from the nanomaterial definition provide in Article 3(z). The Regulation requires that approval of an active substance shall not cover nanomaterials except where explicitly mentioned. The Regulation also requires that where nanomaterials are used in that product, the risk to human health, animal health and the environment has been assessed separately. The Regulation requires additional considerations for nanomaterials, as the simplified authorisation procedure allowed for conventional biocides is not allowed for biocidal products containing nanomaterials. Article 58 of the Regulation requires labelling of all articles treated with biocides. For nanomaterials this obliges the name of all nanomaterials contained in the biocidal products, followed by the word 'nano' in brackets to be on the label.

Organic Production and Labelling of Organic Products

Organic farming and food production have a specific Regulation that was most recently updated and published in 2018 (Miernicki et al. 2019). The Regulation (EU) 2018/848 on organic production and labelling of organic products defines 'engineered nanomaterials' (Tajani and Pavlova 2018) by referring to the definition in the Regulation on novel foods (EU 2015/2283) (Schulz and Schmit 2015).

Article 7 of the Regulation outlines specific principles applicable to the processing of organic food and states (in 7(e)) that the production of processed organic food shall be based, among others, on the exclusion of food containing, or consisting of, engineered nanomaterials. Thus, food products cannot legally be labelled as organic, if they contain engineered nanomaterials.

Nanomaterials Within Medicinal Products (Pharmaceuticals)

The basis for regulating medicinal products in EU is found in a Directive 2001/83/EC on Community code relating to medicinal products for human use (Fontaine and Reynders 2001). This Directive, due to its age, does not mention nanomaterials however, during the second regulatory review of nanomaterials in 2012 (European

Commission 2012), 'The Commission took the view that current legislation on medicinal products allows an appropriate risk/benefit analysis and risk management of nanomaterials.' This was also mentioned in the 2011 definition recommendation which noted the 'special circumstances prevailing in the pharmaceutical sector' and stated that the recommendation should 'not prejudice the use of the term "nano" when defining certain pharmaceuticals and medical devices'. Thus, there is no definition of nanomaterials or nanomedicines in the EU. However, European Medicines Agency (EMA, based in Amsterdam, The Netherlands, since it recent move from London in 2019) does apply a working definition of nanomedicines which outlines the following considerations:

- Purposely designed systems for clinical applications
- At least one component at nano-scale size resulting in definable specific properties and characteristics
  - related to the specific nanotechnology application and characteristics for the intended use (route of administration, dose)
  - associated with the expected clinical advantages of the nano-engineering (e.g. preferential organ/tissue distribution)
- Needs to meet definition as a **medicinal product** according to European legislation.

The EMA produces most of the scientific assessment of medicinal products and as such has produced a number of guidelines related to the use of nanomaterials and nanotechnologies in the sector (European Medicines Agency 2020).

Nanomaterials and Medical Devices

The EU Regulation on Medical Devices, (EU) 2017/746 (Tajani and Borg 2017), provides a definition of nanomaterials (in Article 2(18–21) that is based on the 2011 recommendation. Interestingly, this definition includes 'natural', which potentially could give rise to measurement issues of abrasion from medical devices not intentionally made of nanomaterials, thus giving rise to incidental nanomaterials. Chapter II of Annex I of the Regulation discusses requirements regarding design and manufacture of medical devices. This states that medical 'devices shall be designed and manufactured in such a way as to reduce as far as possible the risks linked to the size and the properties of particles which are or can be released into the patient's or user's body, unless they come into contact with intact skin only. Special attention shall be given to nanomaterials.'

The issue of nanomaterials is then further specified in Chapter III of Annex I where a number of specific rules have to be considered with regards to classification. For nanomaterials, Rule 19, which is based on potential exposure outlines the following for classification:

All devices incorporating or consisting of nanomaterial are classified as:

- class III if they present a high or medium potential for internal exposure;
- class IIb if they present a low potential for internal exposure; and
- class IIa if they present a negligible potential for internal exposure.

The separation into the three classes means that more information will need to be provided where internal human exposure is elevated. A guidance document on how to interpret Rule 19 is currently (autumn 2019) being drafted. It is foreseen to be published later in 2019.

Nanomaterials and Electrical Equipment

The EU has a number of specific sectoral legislations, e.g. on electrical equipment. Two important directives in this area are the RoHS and WEEE Directives, i.e. 'Restriction of the use of certain hazardous substances in electrical and electronic equipment' (European Commission 2020b) and 'Waste Electrical and Electronic Equipment Directive' (European Commission 2020c).

The RoHS directive lays down rules on the restriction of the use of hazardous substances in electrical and electronic equipment. The directive currently in force is referred to as RoHS2 as the first RoHS directive was repealed in 2013. Nanomaterials are mentioned in RoHS2; in the absence of scientific evidence concerning nanomaterials hazardous properties, the European institutions are monitoring them during the process of reviewing Annex II – List of Restricted Substances. The EC monitoring is a constantly ongoing process. The list of restricted substances does not include nanomaterials but upcoming reviews could target them in the future.

The WEEE Directive includes specific mentions of nanomaterials. However, the directive has currently no nano-specific provision. As in the ROHS2 directive, the European Commission reserves the right to amend Annex VII of this Directive to eventually apply selective treatment to nanomaterials contained in Electrical and Electronic Equipment.

Country Specific Registers for Nanomaterials

Mandatory reporting of nanomaterials are required in several EU Members States (EUON 2020). Demands for formalised reporting schemes originate from a number of reasons, ranging from governments' wishes to know what is on their national market to calls for 'the consumer's right to know' made by NGOs and consumer organisations, to market analysts' and policy makers' interest in the extent of innovation through and commercialisation of nanomaterials.

Diverse concepts for information gathering schemes have emerged. Some regulatory authorities sought simple notification of raw materials on the nanoscale as

part of an existing substance- or chemical authorisation process (e.g. Norway and Sweden), while others have set up traceability schemes that are applicable throughout a supply chain and enable the registration of nanomaterials in both raw material form and in final consumer products and waste disposal contexts (e.g. France, Belgium and Denmark).

In 2013 France launched a mandatory declaration for nanomaterials (Anses 2020). Prior to April 30 each year, importers or manufactures of nanomaterials in France must make a declaration for each nanomaterial produced, imported or distributed for the previous calendar year in quantities larger than 100 g.

Denmark, set up its registration of nanomaterials (Virk Indberet 2020a, b) in 2015, Belgium in 2016 (FPS Public Health 2020) and Sweden in 2018 (KEMI 2020). The four schemes are similar in the sense that they all base their definitions of nanomaterial on the 2011 EC recommendation. However, there are also considerable differences between the schemes as they cover different aspects and require different information. Notably, the Danish scheme focuses on substances that are marketed to consumer, and exclude professional use whereas the French, Belgian and Swedish schemes covers professional uses and consumer uses are excluded. In Europe, Norway also has a register where information on nanomaterials should be included (Norwegian Environment Agency 2020).

Innovation to Bring Safe Nanomaterials to the Market: Responsible Research and Innovation (RRI) and Safe(r) by Design (SbD)

The concepts of responsible research and innovation (RRI) and Safe(r) by Design (SbD) have been developed over time to support industries to take an early and active approach to develop new products and innovations that fulfil regulatory requirements for environmental health and safety aspects. It should be recalled that the terms research and innovation are two different processes where research is about generating knowledge, and innovation is about generating new benefits, or (economic) value. Applying RRI means engaging all societal actors early and with the aim of inclusiveness while also addressing mandatory legal aspects and societal relevance and acceptability of research and innovation outcomes (Dreyer et al. 2017). Both RRI and SbD approaches focus on the early stages of innovation and product development where considerations of environmental, health and safety as well as social aspects can have a profound influence of the progressive innovation steps.

Both RRI and SbD in the nanomaterials sphere reflect efforts by policy, research, NGOs and industrial communities to create a framework for the development of novel nanomaterials and nano-enabled products that builds confidence for consumers and industry as well as other communities e.g. public in general, NGOs, workers, along with governments (Rose et al. 2019).

The fast pace at which nanotechnology is currently evolving challenges the regulators' response time for amending legislation and providing a certain pathway for nanomaterials innovation. Ways to minimize this information gap include: (a) industry to reduce uncertainties and risks to humans and the environment from the upstream, early phases of the innovation process (SbD); and (b) regulators to improve anticipation in order that they can facilitate the development of adaptable (safety) regulations that can keep up with the pace of knowledge generation and innovation of MNMs and MNM-enabled products. The underlying fundamental principles in the field to reduce uncertainties are filled by academic research efforts, and by active screening and monitoring of early signs of emerging fields and risks. Regulators should shift from a reactive to a proactive way of working, meaning that it is more efficient to take a proactive approach to ensure that regulations already cover new developments and products than to regulate when products and possible damages have already occurred.

Regulators need to stay up to date regarding innovations and engage in dialogue with academics, innovators, industry and society at large, while recognizing that industry has the main responsibility and legal liability for the safety of their products. Such dialogue serves to share knowledge and insight on how nano-specific characteristics influence exposure and toxicity, and to translate scientific knowledge into action in an efficient manner. A key element of obtaining information and knowledge is dialogue with industry and to obtain willingness of industry to share information early in the innovation process by ensuring confidentiality and protection of commercial interests. This trust forming dialogue and sharing of information needs to be balanced with the potential benefits for society in getting access to innovative products and the public's right to know and in turn, the public's trust of governments. This dialogue and co-creation between Regulators and industry is essential for SbD implementation. Two approaches are discussed here, developed in the EU ProSafe project, which can support the risk management of nanomaterials (Teunenbroek et al. 2017).

The first approach is to change the current risk assessment process to shift it towards a concern-based testing approach. A concern-based approach, for example, put more emphasis on exposure assessments, where a limited exposure (e.g. only under occupational settings) could allow for a simplified risk assessment and risk management as when exposure is low the risk is consequently the risk is reduced. The second approach is the application of SbD). The SbD 'looks at ways to identify, and thus to avoid, potential adverse effects of NMs from the earliest stages of the innovation process onwards' and 'it also holds the potential to create a closer collaboration between product developers and safety scientists as well as among scientists, innovators and regulators who all work together to further the common aims of technology development that will be safe for human health and the environment' (ProSafe Project Office 2017). The basis for SbD for nanomaterials is to create a streamlined innovation process and support industries and academia in a structured manner while still retaining a high protection for human health and the environment. The SbD is also a core part of the Safe Innovation Approach (SIA) which encourages industries to an early integration of safety aspects in the innovation

process as well as applying a regulatory preparedness approach which aim to improve anticipation of regulators so that they are prepared to develop appropriate regulations (Soeteman-Hernandez et al. 2019). SIA aims to be a responsible approach to be used by industry when developing nanomaterials and products while stimulating a proactive dialogue with policymakers and regulators to reduce the gap between appearance and approval of innovative products.

SbD is still a somewhat academic concept and uptake of industries is still in progress, although especially larger companies are sometimes applying the concepts of SbD. The use in small to medium sized companies is still not widespread (Sørensen et al. 2019). For a transition into use by industry Safe by Design must be accessible, robust, effective for achieving required level of protection and cost-effective, tied to guidelines and standards and with a regulatory context. Adoption of RRI and SbD approaches both academia and industry will encourage early and continued assessment of product characteristics from a regulatory health and safety perspective, through the innovation and product development process. This would support efficient use of resources and allow for nanotechnology-enabled products reaching the market faster while retaining a high level of protection for human health and the environment.

References

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses). R-Nano.fr Déclaration des substances à l'état nanoparticulaire [Internet]. R-nano.fr. 2020. Available from: https://www.r-nano.fr/

Barroso J. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Official Journal of the European Union [Internet]. 2011;54(L12):1–93. Available from: http://data.europa.eu/eli/reg/2011/10/oj

Borrell Fontelles J, Nicolai A. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Official Journal of the European Union [Internet]. 2004;47:4–17. Available from: http://data.europa.eu/eli/reg/2004/1935/oj

Buzek J, Ask B. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (Text with EEA relevance). Official Journal of the European Union [Internet]. 2009;52:59–209. Available from: http://data.europa.eu/eli/reg/2009/1223/oj

Buzek J, Dowgielewicz M. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 Text with EEA relevance. Official Journal of the European Union [Internet]. 2011;54(L11);11:18–643. Available from: http://data.europa.eu/eli/reg/2011/1169/oj

Clausen L, Hansen S. The ten decrees of nanomaterials regulations. Nat Nanotechnol [Internet]. 2018;13(9):766–768. Available from: https://doi.org/10.1038/s41565-018-0256-2.

Competent authorities subgroup on nanomaterials. 2nd meeting of the competent authorities subgroup on nanomaterials: registration of nanomaterials in REACH. Doc. CASG Nano/11/2008. Brussels; 2008.

Cox P, Piqué i Camps J. 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 Union [Internet]. 2002;45:1–24. Available from: http://data.europa.eu/eli/reg/2002/178/oj

Dreyer M, Chefneux L, Goldberg A, von Heimburg J, Patrignani N, Schofield M, et al. Responsible innovation: a complementary view from industry with proposals for bridging different perspectives. Sustainability. 2017;9(10):1719.

EU law – EUR-Lex. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC [Internet]. 2006. Available from: https://eur-lex.europa.eu/eli/reg/2006/1907/2020-04-28

EU law – EUR-Lex [Internet]. Eur-lex.europa.eu. 2020. Available from: https://eur-lex.europa.eu/homepage.html

EU treaties | European Union [Internet]. European Union. 2020. Available from: https://europa.eu/european-union/law/treaties_en

EUON. National reporting schemes [Internet]. Euon.echa.europa.eu. 2020. Available from: https://euon.echa.europa.eu/national-reporting-schemes

European Chemicals Agency. Appendix for nanoforms applicable to the Guidance on Registration and substance identification [Internet]. Helsinki: European Chemicals Agency; 2019 p. 1–28. Available from: https://echa.europa.eu/documents/10162/23047722/appendix_nanoforms_draft_to_peg_en.pdf/bb84f0ca-7688-5293-604e-fb43982c7afd

European Commission. Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee – Regulatory aspects of nanomaterials [SEC(2008) 2036] [Internet]. 2008 p. 2–11. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52008DC0366&from=EN

European Commission. Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee Second Regulatory Review on Nanomaterials [Internet]. Brussels; 2012 p. 1–15. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52012DC0572

European Commission. Nanomaterials in REACH and CLP [Internet]. Ec.europa.eu. 2019. Available from: http://ec.europa.eu/environment/chemicals/nanotech/reach-clp/index_en.htm

European Commission. Authorities – REACH – chemicals – environment. [Internet]. Ec.europa.eu. 2020a. Available from: http://ec.europa.eu/environment/chemicals/reach/competent_authorities_en.htm

European Commission. RoHS 2 – electronics waste [Internet]. Ec.europa.eu. 2020b. Available from: http://ec.europa.eu/environment/waste/rohs_eee/legis_en.htm

European Commission. Waste electrical & electronic equipment (WEEE) [Internet]. Ec.europa.eu. 2020c. Available from: http://ec.europa.eu/environment/waste/weee/index_en.htm

European Medicines Agency. Multidisciplinary: nanomedicines [Internet]. European Medicines Agency. 2020. Available from: https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/multidisciplinary/multidisciplinary-nanomedicines

European Parliament [Internet]. European Parliament. 2020. Available from: http://www.europarl.europa.eu/portal/en

Fontaine N, Reynders D. 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. Official

Journal of the European Union [Internet]. 2001;44(L 311):67–128. Available from: http://data.europa.eu/eli/dir/2001/83/oj

FPS Public Health. Register [Internet]. FPS Public Health. 2020. Available from: https://www.health.belgium.be/en/environment/chemical-substances/nanomaterials/register

Guidance on Information Requirements and Chemical Safety Assessment – ECHA [Internet]. Echa.europa.eu. 2020. Available from: https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen H, More S et al. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: part 1, human and animal health. EFSA Journal [Internet]. 2018;16(7):1–95. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/5327

Juncker J-C. Commission Regulation (EU) 2018/1881 of 3 December 2018 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III,VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances [Internet]. 2018;61:1–20. Available from: http://data.europa.eu/eli/reg/2018/1881/oj

KEMI – Swedish Chemical Agency. Rules on reporting to the Products Register [Internet]. Kemi.se. 2020. Available from: https://www.kemi.se/en/rules-and-regulations/rules-applicable-in-sweden-only/rules-on-reporting-to-the-products-register

Miernicki M, Hofmann T, Eisenberger I, von der Kammer F, Praetorius A. Legal and practical challenges in classifying nanomaterials according to regulatory definitions. Nat Nanotechnol [Internet]. 2019;14(3):208–216. Available from: https://doi.org/10.1038/s41565-019-0396-z.

Norwegian Environment Agency. The Product Register [Internet]. Miljødirektoratet/Norwegian Environment Agency. 2020. Available from: http://tema.miljodirektoratet.no/en/Areas-of-activity1/Chemicals/The-Product-Register/

Potocnik J. Commission Recommendation of 18 October 2011 on the definition of nanomaterial Text with EEA relevance. Official Journal of the European Union [Internet] 2011;54:38. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2011.275.01.0038.01.ENG&toc=OJ:L:2011:275:FULL

Pöttering H-G, Le Maire B. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal of the European Union [Internet] 2008;51:1–1355. Available from: https://eur-lex.europa.eu/eli/reg/2008/1272/oj

Pottering H, Le Maire B. Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. Official Journal of the European Union [Internet]. 2008;51:1–6. Available from: http://data.europa.eu/eli/reg/2008/1331/oj

ProSafe Project Office. The ProSafe White Paper: towards a more effective and efficient governance and regulation of nanomaterials [Internet]. 2017 p. 1–47. Available from: https://www.rivm.nl/sites/default/files/2018-11/ProSafe%20White%20Paper%20updated%20version%2020170922.pdf

Rauscher H, Roebben G, Mech A, Gibson P, Kestens V, Linsinger T et al. An overview of concepts and terms used in the European Commission's definition of nanomaterial [Internet]. Publications Office of the European Union; 2019. Available from: https://doi.org/10.2760/459136.

Rose G, Pavlicek A, Gazsó A. Safe-by-design – the early integration of safety aspects in innovation processes [Internet]. Vienna: Institute of Technology Assessment (ITA); 2019 p. 1–6. Available from: http://epub.oeaw.ac.at/0xc1aa5576_0x003aa569.pdf

Schulz M, Schmit N. Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001.

Official Journal of the European Union [Internet]. 2015;58:1–22. Available from: http://data.europa.eu/eli/reg/2015/2283/oj

Schulz M, Wammen N. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Official Journal of the European Union [Internet]. 2012;55(L167):1–128. Available from: http://data.europa.eu/eli/reg/2012/528/oj

Scientific Committee on Consumer Safety SCCS. Guidance on the safety assessment of nanomaterials in cosmetics [Internet]. European Union; 2012 p. 1–62. Available from: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_005.pdf

Soeteman-Hernandez L, Apostolova M, Bekker C, Dekkers S, Grafström R, Groenewold M et al. Safe innovation approach: towards an agile system for dealing with innovations. Mater Today Commun [Internet]. 2019;20:100548. Available from: https://doi.org/10.1016/j.mtcomm.2019.100548.

Sørensen S, Baun A, Burkard M, Dal Maso M, Foss Hansen S, Harrison S et al. Evaluating environmental risk assessment models for nanomaterials according to requirements along the product innovation Stage-Gate process. Environ Sci Nano [Internet]. 2019;6(2):505–518. Available from: https://doi.org/10.1039/C8EN00933C.

Tajani A, Borg I. Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. Official Journal of the European Union [Internet]. 2017;60(L 117):176–332. Available from: http://data.europa.eu/eli/reg/2017/746/oj

Tajani A, Pavlova L. Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007. Official Journal of the European Union [Internet]. 2018;61(L150):1–92. Available from: http://data.europa.eu/eli/reg/2018/848/oj

Teunenbroek T, Baker J, Dijkzeul A. Towards a more effective and efficient governance and regulation of nanomaterials. Part Fibre Toxicol [Internet]. 2017;14(1):1–5. Available from: https://doi.org/10.1186/s12989-017-0235-z.

The European Council [Internet]. Consilium.europa.eu. 2020. Available from: https://www.consilium.europa.eu/en/european-council

The Royal Society. Nanoscience and nanotechnologies: opportunities and uncertainties [Internet]. Plymouth: Latimer Trend Ltd; 2004 p. vii-116. Available from: https://royalsociety.org/~/media/Royal_Society_Content/policy/publications/2004/9693.pdf

Toshkov D. 55 years of EU Legislation. Presentation presented online; 2014.; http://www.dimiter.eu/Eurlex.html

Vassiliou A. Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. Official Journal of the European Union [Internet]. 2009;52(L135):3–11. Available from: http://data.europa.eu/eli/reg/2009/450/oj

Virk Indberet. Homepage [Internet]. Indberet.virk.dk. 2020a. Available from: https://indberet.virk.dk/

Virk Indberet. Nanoproduktregister [Internet]. Indberet.virk.dk. 2020b. Available from: https://indberet.virk.dk/myndigheder/stat/MST/Nanoproduktregister

Index

J. R. Lead et al. (eds.), *Nanotoxicology in Humans and the Environment*, Molecular and Integrative Toxicology, https://doi.org/10.1007/978-3-030-79808-6