Science for Environment Policy
Assessing the environmental safety of manufactured nanomaterials

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# Contents

Executive Summary  5

## 1. Introduction  10
  1.1 General role of policy for the nanomaterials market  10
  1.2 Role of science in policymaking for nanomaterials  14
  1.3 Describing and defining nanomaterials  17
  1.4 Characterisation of nanomaterials  19
  1.5 Rationale for structure of report  20

## 2. State of science of nanomaterials  22
  2.1 History  22
  2.2 Advances in applications and manufacture  22
  2.3 Nanosafety  25
  2.4 Knowledge gaps and challenges  26
  2.5 Current research strategies on nanosafety  28

## 3. Characterisation  31
  3.1 Existing methods for characterisation  31
  3.2 Knowledge gaps and challenges  36
  3.3 Current and future developments  38

## 4. Exposure  39
  4.1 Release of manufactured nanomaterials (MNM)  39
  4.2 Emission and transportation of MNMs  40
  4.3 Environmental exposure to MNMs  41
  4.4 Existing techniques for measurement  42
  4.5 Knowledge gaps  45
  4.6 Current and future developments: nanomaterials exposure under project MARINA  50
## Contents continued

5. **Hazard: toxicology and ecotoxicology**  
   5.1 Toxicology  
   5.2 Ecotoxicology  
   5.3 Nanoeotoxicology  
   5.4 Current understanding of nanoeotoxicology  
   5.5 Species sensitivity distributions  
   5.6 Knowledge Gaps  
   5.7 Current developments:  

6. **Risk assessment**  
   6.1 Existing risk assessment methodologies (Rickerby *et al.* 2015; Rio-Echevarria & Rickerby 2015)  
   6.2 The MARINA risk assessment strategy (Bos *et al.* 2015)  
   6.3 Life cycle analysis  
   6.4 Knowledge gaps  
   6.5 Current developments  

7. **Current and future challenges for the science-policy interface**  
   7.1 Nanomaterials on the market  
   7.2 Third-generation MNMs and beyond  
   7.3 Nanomaterials as part of a product  
   7.4 Closing knowledge gaps  
   7.5 Meeting the challenges  

**References**
Executive summary

Engineering at the nanoscale (one million to ten thousand times smaller than a millimetre; i.e. 1 to 100 nanometres) brings the promise of radical technological development — clean energy, highly effective medicines and space travel. But technology at this scale brings its own safety challenges. This In-depth Report shows that, despite early fears, nano-sized particles are not inherently more toxic than larger particles, however, differences between them may be notable and new insights are still being provided by research. The effects of nanoparticles on humans and the environment are complex and vary based on particle properties as well as chemical toxicity. This Report brings together the latest science on environmental safety considerations specific to manufactured nanoscale materials, and the possible implications for policy and research.
The history of nanotechnology already features an evolution through several generations. First generation nanotechnologies (pre 2005) generally refers to nanotechnology already on the market, either as individual nanomaterials, or nanoparticles incorporated into other materials, such as films or composites; second generation nanotechnologies (2005-2010) are characterised by nanoscale elements that serve as the functional structure, such as electronics featuring individual nanowires. From 2010 onward there has been more research and development of third generation nanotechnologies, which are characterised by their multi-scale architecture (i.e. involving macro-, meso-, micro- and nano-scales together) and three-dimensionality, for applications like biosensors or drug-delivery technologies modelled on biological templates. Post 2015, the fourth generation are anticipated to utilise ‘molecular manufacturing’: achieving multi-functionality and control of function at a molecular level.

The unproven potential of nanotechnology is huge: stronger, more efficient, cleaner and vastly more compact materials could make complex products that
have every atom in a calculated placement. Suggested applications range from cheap, tiny sensors, mechanisms and implantable devices allowing continuous health monitoring or semi-automated treatment of disease, to cheap and widespread supercomputers that fit inside a few cubic millimetres, to the recovery and reuse of clean water globally, to large solar energy efficiency improvements. Some of the more distant projections for this technology include affordable, globally distributed energy and computer hardware systems, new agricultural or industrial ‘revolutions’, advanced robotics, space travel, and the ability for tiny nanomanufacturing systems to build complex products easily and cheaply — and even to build other nanomachines or self-replicate.

The proven potential is considerably smaller, but ever increasing and diversifying; many researchers are also keen to ‘future-proof’ their ideas with ecologically viable solutions. There is already evidence that nanotechnologies can reduce polluting manufacturing byproducts, increase the efficiency of solar cells and windmill blades, and clean up contaminated groundwater, oils spills and airborne volatile organic compounds. Other examples of nanotechnologies include nanomaterials that are able to withstand extreme heat and tension, drug-delivery systems that can deliver drugs straight into disease or cancer cells, nanolithography which uses electron beams to etch silk proteins into finely detailed patterns (e.g. for microchips) — and pomegranate-shaped batteries, storing twice the energy of a regular lithium-ion battery, which can be made using the silicon from recycled rice husks.¹

However, in contrast to the fast-moving research and development into new properties, materials and possibilities of nanotechnologies, the research to support comprehensive risk assessments is often lagging behind. There are concerns about the larger surface areas and different shapes and interactivity that some MNMs possess – which may cause them to react differently to their macro, meso or micro counterparts. Due to their small size, biological barriers are not always an obstacle for nanoparticles — such as the blood-brain barrier, or the placental barrier between mother and foetus.

Practically speaking, measuring and quantifying MNMs in real situations is a challenge. In most cases they are not directly detectable by regular analytic methods due to their very low concentration in the studied organism and/or environment. And, even if detected, there are difficulties in differentiating between naturally occurring nanomaterials and the MNMs. Additionally, most in vitro and in vivo studies conducted so far are only short term studies, while impacts on human health and the environment are, in many cases, more likely to occur after long(er)-term exposure. Consequently, there is an urgent need for long-term exposure studies.

As for all chemicals, comprehensive individual testing of each nanomaterial would always be preferred, but its feasibility is questionable, financially and ethically.

¹ For more details of these developments, see Science for Environment Policy’s Thematic Issue on Nanomaterials’ Functionality: http://ec.europa.eu/environment/integration/research/newsalert/pdf/nanomaterials_functionality_48si_en.pdf
The equipment and time are expensive, and the drive to reduce animal testing combined with the increasing number of MNM applications on the market or close to commercialisation suggests that new methods to assess exposure and hazard will be needed. Attempts to group nanomaterials into classes with similar properties, which are regulated similarly under legislation, are ongoing; these approaches still rely strongly on the data from high-quality scientific studies that underpin the assessments. A debate around categorisation, grouping and individual assessment of nanomaterials is ongoing: the European Commission’s (EC) Directorate General (DG) ENV organised an Organisation for Economic Cooperation and Development (OECD) expert meeting in April 2016 on ‘Grouping and Read-Across for the Hazard Assessment of Manufactured Nanomaterials’. It included several proposals for different ways to group nanomaterials and how to extend both the OECD’s guidance on grouping of chemicals and REACH (Registration, Evaluation, Authorisation & restriction of Chemicals Regulation EC 1907/2006) to nanomaterials. The expert meeting reached consensus conclusions for the main issues, but the finer details and their application to different materials still need more work.

For future work, there is a clear need to pool knowledge and resources and optimise the application of all existing data for hazard, exposure and risk assessments. Environmental risks for first-generation nanomaterials have not yet been fully characterised, due to data gaps in how processes in the environment may transform MNMs, for example, although there is a clear method and process ongoing to collect data on these. For next-generation nanomaterials data is very scarce, even regarding their physico-chemical properties and how these properties transform over time and as MNMs are transported through the environment. Systems toxicology, which applies some of the computational approaches of systems biology to toxicological questions, may provide good insights and answers to some of the questions.

The current EU approach to regulation of chemicals, REACH, places the burden of proof for safe use on Industry. Under REACH, businesses must identify and manage the risks of any substances that they intend to manufacture/import and/or market, including demonstration of their safety and communicate relevant risk management measures to downstream users. Substances can be restricted or banned, if the risks are unacceptable. In the relatively unchartered territory of next-generation nanomaterials, one of the main challenges for effective regulation is ensuring sufficient robustness of studies and data. Given the current lack of methods and techniques for determining all hazards and exposures of these materials (some for which their transformation potential is a defining feature of their
function), it is imperative to ensure current methods for safety assessment do not create loopholes, e.g. by remaining static and possibly inflexible while the field advances. Also, in the case of nanomaterials the burden of proof of safe use will remain with Industry: companies should prepare and publish their evidence on safety for each MNM manufactured/imported and/or marketed.

To go further, if Industry needs new/updated test guidelines to prove the safety of a MNM then it is perhaps their responsibility to propose and validate one — or to validate an existing test guideline for application to MNMs (noting that regulatory test guidelines are agreed within the OECD test guidelines programme (TGP)). However, this raises the question of whether a company can be said to have sufficiently validated a test just by committing sufficient time and resources to validation — or whether there are actually some objective standards by which test validation should be judged; the OECD TGP has developed guidance on this. Another way industries can handle the burden of proof is to demonstrate the relevance of existing data or a grouping approach.

There are reasons to be cautious in both cases, however. Even with the older generations of nanomaterials (for which we have more data), any subsequent discovery of unforeseen biological or physicochemical effects leaves the possibility open that large quantities of scientific data, gathered in ignorance of such effects, become void. Because MNMs may exhibit such unforeseen effects, additional regulatory requirements beyond those for comparable macro-materials could be relevant. In all cases, companies manufacturing/importing the nanoforms of the same chemical need to co-operate on REACH registration. With differing forms and properties of manufactured nanomaterials, even within one chemical composition, blanket generalisations — about their transformation potential, risks to the environment and their fate and behaviour in the environment — are not practicable, or advisable.

If nanomaterial production and diversity were to reach the scale implied by the nanotechnology visionaries, identifying and managing possible risks early in the development stages is the only way the field can advance responsibly. At present it can seem a challenging task for Industry to show their compliance with REACH (especially for smaller manufacturing companies presently preparing for the May 2018 registration deadline for substances produced at 1–100 tonnes per annum). However, there are early indications that a more pre-emptive research- and industry-led process for demonstrating compliance could become a more efficient way of developing new nanomaterials.

There is a growing interest — supported by several EU projects, such as the NanoSafety Cluster Compendium, NANoREG and NANoREG² — in a ‘Safe-by-Design’ (SbD) approach, which posits that any health or safety risks should be ‘designed out’ in the development phases, based on data to be curated and shared systematically. SbD would represent a further shift towards the burden of proof approach found under REACH. However, this will also require increased cooperation between different parts of the relevant value chain — between researchers, designers, developers, distributors, potential end-users and regulators. To create such a culture for the development of innovative materials, ‘scoping’ stages would need to be more carefully planned and better funded. In addition, more incentives would need to exist to publish and publicise negative results. Indeed, there is a need for platforms and networks, such as the European Observatory for Nanomaterials, that will enable unprecedentedly accessible data and collaboration between businesses, researchers, decisionmakers and assessment experts.

4 Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. OECD series on testing and assessment No. 34.
5 For details of EU projects, see the European NanoSafety Cluster Compendium: https://www.nanosafetycluster.eu/home/european-nanosafety-cluster-compendium.html
1. Introduction

This In-depth Report provides a summary of current scientific progress of the techniques and methods to assess nanomaterial safety, particularly in relation to the environment. The aim is to present the most promising strategies and most significant challenges of nanomaterial characterisation, exposure, fate and behaviour, ecotoxicological hazard and risk assessment, and examples and case studies of both the scientific developments and the knowledge gaps. It is intended to complement the work of those developing the next generation of nanotesting and assessment, and to support a more systematic, intelligent development for nanotechnologies, and the significant benefits they could provide. In the last decade nanotechnology and the use of nanomaterials has become the subject of much debate. Under a general definition, nanomaterials are chemicals or materials that have constituents with nanoscale proportions, which is one billionth of a metre or 0.000 000 001 metres. This is 10 000 times smaller than the diameter of a human hair. Compared to the same material without nanoscale features, nanomaterials may exhibit novel characteristics, such as increased strength, chemical reactivity or conductivity.

Although the debate about them is relatively recent, nanomaterials are not new but have existed in nature before they were named, defined and discussed. For millions of years they have formed within soils, the oceans, and the atmosphere, for example (see Box 1.1 for specific examples).

Even man-made nanomaterials are not as novel as might be expected. As long ago as the 4th century CE, Romans used nano-sized particles of gold and silver in the Lycurgus Cup so that different positions of the source of light and the observer seemingly change the colour of the cup.

The increase in debate has been fuelled by the rise in the production and use of manufactured nanomaterials (MNMs), alongside a lack of scientific knowledge around the potential impacts of MNMs.

MNMs are often designed to accomplish a particular purpose, taking advantage of the fact that materials at the nanoscale have different properties than their larger-scale counterparts. They might be stronger or lighter, more reactive or conduct electricity more effectively. By engineering nanomaterials people can harness these properties to make valuable new products or processes. Changing the form of a nanomaterial can produce a material with an entirely new property (i.e. a new nanomaterial); however, we often refer to a group of such materials developed from the same chemical substance as a nanomaterial (e.g. nanoTiO₂) that is available in different (nano)forms. The same properties that make nanomaterials so useful may also lead to risks to the environment and raise health and safety concerns.

Nanomaterials are not necessarily more, or less, hazardous than non-nano chemicals, but some may have different environmental or human health impacts than those of equivalent bulk materials. As such there may need to be specific provisions in regulations to ensure these differences in impacts are addressed when carrying out risk assessment and management (Broomfield et al. 2016). There are an increasing number of products entering the market that are either produced by nanotechnology or that contain nanomaterials. Current applications include healthcare (in targeted drug delivery, regenerative medicine, and diagnostics), electronics, cosmetics, textiles, information technology and environmental protection. For example, nanosilver is applied in a range of products, including washing machines, socks, food packaging, wound dressings and food supplements.

As the range and number of products continue to increase so does the need for effective risk assessment methods to ensure safe use of nanomaterials. Any risk assessment has a degree of uncertainty, and the key lies in finding an acceptable level of uncertainty which ensures that the nanomaterials on the market are safe without requiring any level of cautiousness preventing us from reaping the possible technological, social and environmental benefits that nanomaterials can offer. There should be thorough and comprehensive hazard testing to determine any adverse effect(s) on humans and the environment, enabling also the establishment of levels of safe exposure before application at a mass-market scale.

1.1 General role of policy for the nanomaterials market

Policy plays an important role in maximising the benefits of MNMs to society while balancing and minimising risks. At a global level estimates for the size of the nanomaterials market vary significantly; numerous reports are released and sold by market research companies, with varying values. Mordor Intelligence (2017) valued the global nanomaterials market at $4097.17 million (approx. €3698.98 million) in 2015, expecting it to reach $11 252.76 by 2020 (approx. €10159.13); in 2015, BCC Research estimated it would reach $64.2 billion (approx. €57.96) by 2019. North America is the largest market in 2014 followed by Europe. In 2012, a European Commission Communication estimated the global value of nanomaterials at 11 million tonnes per year, with a market value of €20 billion. Direct EU employment
in the nanomaterial sector was estimated at 300,000 to 400,000 in the EU, and products underpinned by nanotechnology were forecast to grow from a volume of €200 billion in 2009 to €2 trillion by 2015. It is unclear from the available literature whether, if any of, these projections have been borne out.

Manufacturers producing nanomaterials in volumes of <1 tonnes per year in the European Union do not need to report production. So far, some regulations at an EU level include specific provisions regarding nanoamaterials; others do not include specific provisions, but have so far applied general provisions on chemicals, which are extended by recommendations, specific reviews and technical guidance specifically addressing nanomaterials (see Box 1.2). The more generic EU policies such as the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and the CLP (Classification, Labelling and Packaging) regulations aim to ensure that manufacturers, importers and downstream users manufacture, market or use substances in a way that does not adversely affect human health or the environment. REACH and CLP address chemicals in general, and hence nanomaterials are also covered by these regulations and risk assessment is expected to cover all forms of a substance placed on the market (2nd regulatory review). There are currently no explicit requirements for nanomaterials under REACH or the CLP regulation.

There are some EU regulations that explicitly address nanomaterials, with a few where the objective of the specific provisions is to ensure that nano-enabled products are risk-assessed, taking into explicit consideration the fact that nanomaterials are used. Risk assessments would normally cover the restricted scope of use covered by each regulation.

Food legislation
- **Regulation (EU) 2015/2283** on novel foods (‘novels food Regulation’), where one the categories of novel foods are engineered nanomaterials; authorisation system is applied
- **Regulation (EC) No 450/2009** on active and intelligent materials intended to be in contact with food. Position on the list of authorised substances (including nanomaterials) is required unless some derogations apply; nanomaterials are not excluded by general derogation to active substances that are not in direct contact with the food
- **Regulation (EU) No 10/2011** on plastic materials and articles intended to come into contact with food; nanomaterials must be explicitly authorised, several have been authorised already
- **Regulation (EU) No 1169/2011** on the provision of food information to consumers; does not include explicit risk assessment provisions; but nanomaterial ingredients need to be explicitly labelled with (nano) in parentheses (implies consideration has been given to the ingredient being a nanomaterial)
- **Regulation No 609/2013** on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control; for nanomaterials, compliance with requirements on the basis of adequate methods must be explicitly demonstrated

Cosmetics Products Regulation (EC) No 1223/2009; a notification procedure is expected for nanomaterials (which then need to be explicitly authorised as ingredients as well as labelled (nano) in the ingredient list. The European Commission may request an opinion on safe use of that nanomaterial in cosmetic products from the Scientific Committee on...
Consumer Safety (SCCS) which reviews submitted toxicological data. The data submitted usually has to follow the SCCS-guidelines.

The Biocidal Products Regulation (EU) No 528/2012; regulation specifies that approval of active substance does not cover its nanoform, which requires specific assessment and authorisation. Also other nanomaterials used in biocidal products and co-formulants must be considered. When providing information, consideration on the appropriateness of the methods employed must be indicated, and a 5-year reporting of the use of nanomaterial is envisioned.

Regulation (EC) No 66/2010 on the EU Ecolabel; no consistent approach in the coverage of nanomaterials under the different Ecolabel criteria, but specific decisions require the applicants to demonstrate that all forms of substances (i.e. including nanoforms) used are not falling (as supported by relevant data) under certain categories of hazardous substances under CLP and are not substances of very high concern under REACH.

Recommendation and guidance

- Scientific Basis for the Definition of the Term “nanomaterial” Approved by the SCENIHR by written procedure on 8 December 2010.
- Scientific committee on emerging and newly identified health risks (SCENIHR) provides opinions on emerging or newly-identified health and environmental risks and on broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. SCENIHR has adopted two opinions:
  - Final opinion on Nanosilver: safety, health and environmental effects and role in antimicrobial resistance 11 June 2014
  - Final opinion on Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices 06 January 2015

Most of the current regulations were designed to address risks of chemicals in general. For some nanomaterials currently being developed, entering commercial markets, or already on the market (i.e. any being produced in quantities of less that 1 tonne/year/manufacturer/importer, and hence not covered by REACH), it is not known how they behave when released into the environment. It is important for regulation to enable legislators to identify and address potential risks of nanomaterials on the market, being developed now and in the future (Broomfield et al. 2016). The challenge is to build a regulatory system that is flexible enough to deal with new technologies and requirements.
1.2 Role of science in policymaking for nanomaterials

Science has an important role to play in informing policy on nanomaterials. Figure 1.1 shows the different ways science can support regulation and below are four more specific examples of how it can inform and support policymaking.

1. One challenge for policy is the development of methods that reliably identify, characterise and quantify nanomaterials both as standalone substances and in various products, or combined with various media. Science can help provide techniques and guidance on measurement to determine what should be considered nanomaterials and how they vary on important properties (see Section 3.1.2).

2. Nanomaterials have a tendency to transform during their life cycle, for example nanoparticles may bind to other kinds of materials, coatings may be formed or disintegrated and properties may change due to chemical reactions, such as oxidation. As such, a nanomaterial that is defined as nano before its use in products may no longer be identified as a nanomaterial by the time it is part of the product or when the product is disposed of (Bleeker et al. 2013). Thorough scientific research is needed to provide more information on the details of these changes and the implications this may have for their impacts on health or the environment. Currently projects such as NanoValid and MARINA have investigated techniques to better identify and describe these changes.

3. Perhaps science’s most important and urgent role is to help provide effective, evidence-based methods to assess risk and safety. The risk assessment of manufactured nanomaterials has become the focus of increasing attention firstly because of the increasing amount of MNMs and secondly because of the lack of knowledge about the impacts. The European Commission is supporting research into the potential impact of nanomaterials and nanotechnology based products on human health and the environment through a number of projects (see Box 1.3). It is hoped that by addressing the knowledge gaps (e.g. toxicity thresholds, transformation processes), the nanomaterials policy can be implemented successfully. In order to assess risk and safety it is necessary to know more about the transformations mentioned in point 2 and develop better measurement techniques as discussed in point 1. As is the case for chemicals in general, science can not provide one definitive answer or solution to assessing risk. However science can inform the tools in this area and help ensure they are effective at assessing the risk of each MNM that is placed on the market.

4. Moreover, science can provide information on the value added to society by nanomaterials in terms of their environmental, medical and product development applications. This is useful when making decisions about how to manage a nanomaterial if there are risk implications. Social science can also help to evaluate public opinion on nanomaterials, which is vital to legislation.
Historically there has tended to be more investment in the research and development of nanomaterials than in the safety of nanomaterials. The total spent on nanosafety projects under FP7 from 2007-2012 was approximately €106m. For comparison, the total FP7 funding for nanoscience and nanotechnologies between 2007 and 2011 was over €2.5bn. There have been reports that, over the period of FP7 (2007-2013), nanotechnology research funding in Europe (boosted in part by the large budget of RusNano) exceeded €29bn.

Improving our scientific understanding of environmental impact of nanomaterials is a worldwide concern and different initiatives have started in several countries. These include the European Union’s Seventh Framework Programme (FP7) and Horizon 2020 Framework Programme on nanotechnology and the US National Nanotechnology Initiative (NNI). There is a need to develop a common framework by providing definitions, global standards, responsible development and test methods (Kah & Hofmann, 2015). This is a priority of the EU delegation in the Organisation for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterials (WPMN).

There has been criticism that the process to convert science into meaningful policy is too slow, either in terms of the modification of

Box 2. EU-supported research projects on nanosafety

The EU NanoSafety Cluster is a DG Research and Innovation initiative to provide a forum for discussion, problem solving and planning R&D activities in Europe and to maximise the synergies between the existing European (FP7 and H2020) and national research projects addressing all aspects of nanosafety including toxicology, ecotoxicology, exposure assessment, mechanisms of interaction, risk assessment and standardisation.

Below are examples of the projects most relevant to this report.

**NANoReg** aims to provide legislators with a set of tools for risk assessment and decisionmaking instruments for the short to medium term and develop long-term, new testing strategies adapted to a high number of nanomaterials where many factors can affect their environmental and health impact. [http://www.nanoreg.eu/]

**NanoDefine** will develop an integrated approach based on validated and standardised methods to support the implementation of the EC recommendation for a definition of nanomaterial. [http://www.nanodefine.eu/]

**NanoMILE** intends to identify the critical properties (physico-chemical descriptors) that confer the ability to induce harm in biological systems, which supports a ‘safety by design’ approach in nanomaterial production. It is developing a set of documented protocols; MNMs libraries on structure and transformation in contact with living systems and mechanistic and quantatitive descriptions of properties; a handbook of best practice. [http://nanomile.eu-vri.eu/home.aspx?lan=230&tab=2657&itm=2657&pag=1606#bl4788]

**Project Managing Risks of Nanoparticles (MARINA)** has developed reference methods for managing the risk of engineered nanoparticles and engineered nanomaterials. [http://www.marina-fp7.eu/]

**NanoValid** is developing reference methods for hazard identification, risk assessment and Life Cycle Assessment (LCA) of engineered nanomaterials. [http://www.nanovalid.eu/]

**QualityNano** is a pan-European infrastructure for Quality in nanomaterials safety testing. [http://www.qualitynano.eu/]

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11 For more information [http://www.nanovalid.eu/]
12 Project Managing Risks of Nanoparticles (MARINA) see [http://www.marina-fp7.eu/]
### Table 1: Commercial applications of a selection of better-known nanomaterials from the DaNa website

<table>
<thead>
<tr>
<th>Name</th>
<th>Main applications</th>
</tr>
</thead>
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| **Fullerenes (Buckyballs, C_{60}, nanoparticles)** | • Plastics and other composite materials. Sports equipment (e.g. in golf clubs, badminton and tennis rackets C_{60} molecules are integrated into shafts and frames to obtain very thin-walled, lightweight robust carbon structures).  
• Anti-ageing cosmetic products.  
• Lubricants.  
• Not currently marketed in Europe. |
| **Titanium Dioxide (TiO_{2}), nano and larger particles** | • Used in high-factor sunscreens (especially sun protection factor +25), and cosmetics.  
• Textile fibres  
• Wood preservatives.  
• Used as coatings and pigments providing whiteness and opacity to papers, paints, varnishes, plastics, polymers, printing inks, fibres, rubber, glass and glass ceramics, tiles, flagstones and solar panels.  
• Food additive number E171.  |
| **Silicon Dioxide (SiO_{2}), including quartz, (i.e. crystalline and micron-sized) and amorphous silica (nano-sized), particles** | • In tyre manufacturing they are used alongside carbon black, to reduce tyre roll and fuel consumption.  
• Water repellent for cotton in textile industry.  
• Abrasive agent in the electronics industry.  
• A base material for the glass, optical and building industries.  
• Provides transparent hardness and scratch resistance of surface coatings.  
• Prevents clogging in powdery foods; food additive E551.  
• A component of medicinal clay.  
• Used in pharmaceuticals, such as tablets, suppositories, gels and creams.  
• Manufacturing concrete and other building materials.  
• As filters and dessicants.  |
| **Zinc Oxide (ZnO), nano and larger particles** | • Used in high-factor sunscreens with sun protection factors above 25.  
• Used in multifunctional textiles, with a range of different uses.  
• Clear varnishes for wood products and furniture.  
• In transparent plastics and plastic films (plastic glasses).  
• Used to promote process of vulcanization in rubber tyre production.  
• Added to cements and paints to increase water resistance or to enhance smoothing properties.  
• Electronic components and semi-conductors (transparent conductive layers) in blue light-emitting diodes, liquid-crystal screens, varistors, and thin-film solar cells.  
• Used in antiseptic and astringent zinc salts, zinc ointments, zinc pastes, adhesive tapes, and bandages for skin and wound treatment, by the pharmaceutical industry.  
• Used as a catalyst and in scientific R&D.  |

13 Information in Table 1 is intended to be indicative; it is sourced from the information on the DaNa website, which is based on a review of the available literature. Due to the different treatments and experimental designs in different scientific studies, the information in this table may not be comprehensive or categorically consistent.  
14 TiO_{2} is also used as catalysts, electric conductors and chemical intermediates, and as technical pure titanium for Scientific R&D.  
15 SiO_{2} is also used to clarify beer and as an abrasive agent in toothpastes.  
16 Current uses of ZnO in textiles include as a broad-spectrum UV-absorber to protect from the sun ([http://nanophase.com/markets/textiles](http://nanophase.com/markets/textiles)). Some proposed uses of Zinc Oxide nanoparticles on textiles are as a flexible working electrode for the detection of aldicarb (ALD) pesticide, as a photocatalyst for the degradation of organic molecules, and as antibacterial agents against *Escherichia coli* (Hatamie et al., 2015).
Carbon nanotubes, nanoparticles

- Used in the automotive industry, for lightweight construction, or for the production of sports equipment, such as bicycle frames.
- Added to plastics in electronics.
- Can be conducting, semi-conducting or isolating in nature according to production methods.\(^1\)

Nanoclays, nanoparticles

- Mainly used in flame retardant products.
- Used in electrical and electronic industries.
- Additives in food contact materials – as food packaging and films (not approved in the EU).
- Used to reduce radioactive contamination (binds caesium).
- Used in cat litter.

Silver (nano-Ag), nano and larger particles

- Used in plastic packaging to keep food fresh.
- Used for antibacterial properties in e.g. wound plasters and bandages, implants, socks and other clothing, creams and lotions.
- Formed in situ: Traditional ‘lunar caustic’ silver nitrate sticks used to etch off warts via the release of corrosive nitric acid were a source of silver nanomaterials.
- Food additive E174.\(^2\)

Source: DaNa 2.0: Information about nanomaterials and their safety assessment: http://nanopartikel.info/en/nanoinfo

regulations (for example, specific provisions under REACH announced by the European Commission in 2012) or in terms of regulatory action on individual materials (for example, in 2012, Hansen and Baun commented that the evidence for nanosilver’s harmful effects was already strong enough and that action at EU level should be taken without delay) (Hansen and Baun, 2012).

### 1.3 Describing and defining nanomaterials

Nanomaterials can take many shapes such as rods, fibres, tubes or fullerenes (geometric ball-shaped structures) but, as their name ‘nanomaterial’ suggests, what ultimately defines them is their size.

#### 1.3.1 Debate around defining nanomaterials only in terms of size

Some researchers have argued that a ‘one size fits all’ definition of nanomaterials will not capture the important considerations for risk assessment (Maynard 2011) and that properties, other than size, influence risk, for example porosity, particle shape and surface area.

The scientific community has agreed that the aspect that all nanomaterials have in common is size, a definition of “nanomaterial” should be based on size, and thus there is a need to understand if and how to address nanomaterials specifically in legislation to ensure an adequate risk assessment to protect humans and the environment. Clear, understandable and evidence-based regulation and guidance is necessary for manufacturers to be able to take appropriate action. The definition used in policy is discussed in Section 1.3.2.

#### 1.3.2 Policy definition of nanomaterials

Materials defined as ‘nano’ are not inherently hazardous. If they are hazardous, the hazards may be equal to their corresponding bulk material, if existing. However, nanomaterials may have a wider and uncertain range of potential effects, due to greater mobility based on their

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\(^1\) Carbon nanotubes may also have uses in textiles and flame-retardant products.

\(^2\) Nano silver can also be used in photovoltaics and optical spectroscopy for light harvesting and can be used to enhance imaging, sensing and biosensing technologies, and in conductive coatings, inks and composites.
small size, when compared with macro-sized materials. The primary purpose of a definition in a policy context is to identify materials for which there may need to be special provisions, for example, in terms of risk assessment or ingredient labelling. This is not to say that materials defined as nano are hazardous but that certain considerations, such as their structure and size, may be needed in their assessment.

In 2011 the European Commission published a Recommendation on the definition of nanomaterial (2011/696/EU), suggesting to define nanomaterials according to a size range, i.e. 1nm-100nm, as this is the range within which the majority of specific nanoscale phenomena occur (Auffan et al., 2009). The Recommendation also clarifies the fraction of particles that need to be in this size range for a material to be classified as nano.

The European Commission’s Recommendation for a definition (Box 3) was informed by an opinion from the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). The SCENIHR is an independent Scientific Committee that provides the Commission with scientific advice when preparing policy and proposals relating to consumer safety, public health and the environment.

The SCENIHR opinion (SCENIHR, 2010) evaluated possible properties other than size that could be used to define nanomaterials. For example, surface area, surface modification and 16 important physical and chemical characteristics proposed by the OECD WPMN (OECD 2008). The SCENIHR acknowledged that all of these properties need to be considered for the purpose of risk assessment but none of them appears to be universally applicable as a criterion for a definition. The committee concluded that size is universally applicable to all nanomaterials and a defined size range would facilitate a uniform interpretation.

The SCENIHR suggested that a definition for regulatory purposes should also include the size distribution, that is the percentage or ratio of the particles that are nano compared to those that are bulk in the material. As described in Box 3, size distribution is included in the definition (see also Section 3.1.1).

More recently the European Union’s in-house science service, the Joint Research Center (JRC), has gathered and analysed feedback from those stakeholders on

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**Box 3. Definition of Nanomaterial according to European Commission Regulation 2011/696/EU**

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.

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

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19 Both agglomerates and aggregates are ‘collections’ of particles. For most definitions, in agglomerates the particles are joined loosely together and can be broken relatively easily, whereas, in aggregates, there is a definite pattern of particles or molecules.

20 Agglomeration/ aggregation; Water solubility/ Dispersability; Crystalline phase; Dustiness; Crystallite size; Representative Electron Microscopy (TEM) picture(s); Particle size distribution – dry and in relevant media; Specific surface area; Zeta potential (surface charge); Surface chemistry (where appropriate); Photocatalytic activity; Pour density; Porosity; Octanol-water partition coefficient, where relevant; Redox potential; Radical formation potential. (OECD, 2008).
their experience of working with the definition. This has looked at the experience of implementing the definition, the different elements of the definition itself and the clarity of the wording around the definition. This analysis suggests that some small changes could be made to the definition alongside guidelines on how to implement it.

More specifically, it has published three reports: the first one collects information concerning experience, the second one analyses this information and the third one provides options and recommendations for change for the recommended definition, based on the content of the two first reports. The reports are:

- **A compilation of information concerning experience with the definition** (EUR 26567 EN)
- **An assessment of collected information concerning the experience with the definition** (EUR 26744 EN)
- **The scientific-technical considerations to clarify the definition and to facilitate its implementation** (EUR 27240 EN)

According to the reports, there is no scientific need to consider major changes, but rather minor clarifications in the text and ways to facilitate the implementation of the definition would be helpful.

Surface area is another property that has been proposed as a defining characteristic, for example in the form: total surface area per unit of mass, i.e. volume-specific surface area (VSSA), specifically 60m²/cm³ (EC Recommendation, 2011/696/EU). However, for agglomerates, aggregates, porous materials, or materials with coatings, measurement of the surface area may be misleading. Where nanomaterials are formed in aggregates or agglomerates, these materials would exhibit a smaller surface area than the equivalent volume of individuated particles. However, in some cases, agglomerates and aggregates made of nano-scale constituents can retain the nano-specific properties and phenomena that pertain to individuated nanoparticles: for example, TiO₂ preserves its photocatalytic properties and UV-absorption characteristics when aggregated.

The Commission’s **Recommendation (2011/696/EU)** may be considered as somewhat confusing about whether or not a VSSA of higher than 60m²/cm³ always means that a material should be classified as a nanomaterial (Rauscher et al., 2015). Specifically, according to the recommendation, VSSA can be used to determine compliance with the definition of nanomaterial, but cannot be used to show that a material is not a nanomaterial. Most cases would be correctly identified: a measurement of less than 60m²/cm³ would trigger a definition in terms of size in any case. But some have noted that some larger particles, for example a large particle with a high-surface-area coating, may be ‘incorrectly’ classified as a nanomaterial. Rauscher et al. (2015) recommend that VSSA could be retained as a proxy or additional criterion to the definition, but only if the wording is adjusted to prevent this confusion, or that it should be used as guidance only – as one screening method among several for implementation of the definition. They also recommend ensuring consistency of definition across all legislation and therefore sectors.

It is planned that the main elements of the actual definition, in terms of size and particle number size distribution, and addressing natural, incidental particles as well as manufactured particles, will remain the same. The JRC however recommends several clarifications.

### 1.4 Characterisation of nanomaterials

Once a material has been defined as nano it is important to recognise how nanomaterials differ from each other, i.e. to characterise them. Nanomaterials vary widely, not only between different materials but between different forms of the same chemical composition due to variation in coatings, shapes, impurities, degree of agglomeration or aggregation etc. Although there may be data available on particles that are similar...
in terms of size or composition these data are not necessarily applicable to all these particles because of differences in other physical and chemical properties (Rio-Echevarria & Rickerby 2015). Describing these differences in physical and chemical properties is known as characterisation.

Characterisation is undertaken to provide an accurate and quantitative description of a nanomaterial, which is sufficient for the required purpose. However, the description should not be impractical to implement, whether it is needed for scientific or regulatory purposes (Pettitt & Lead 2013). Characterisation of MNMs for the purpose of assessing safety is covered in more detail in section 3.0.

Even if two nanomaterials differ with respect to a few properties, it is important to clarify when two materials are equivalent in terms of their hazard and exposure profiles and when they should be considered separate, leading to an understanding of the needs for data specific to each nanoform of the same chemical composition. The differences between nanomaterials needs to be acknowledged in regulation, and no unwarranted assumptions about one nanomaterial’s similarity with other nanomaterials and chemicals should be made.

1.5 Rationale for structure of Report

This report will focus on science’s contribution to the techniques and methods to assess risk and safety As mentioned previously research into risk and safety also relies on identifying nanomaterials and on understanding the life cycle transformation of nanomaterials. It will provide a summary of the state of the science in this area with examples and case studies of both the scientific developments and the knowledge gaps.
Research assessing the safety of nanomaterials is in a constant state of development to catch up with the fast advances in nanomaterial development.

Applying and adapting existing risk assessment methodologies is a challenge; due to their size and associated properties, MNMs may require additional testing beyond the standard suite of tests used for other chemicals, to ensure that the impact on human health and the environment is fully understood. Accurate risk assessments may also require adaptation of current test methods to identify nanospecific issues. There are also challenges in characterising individual MNMs and actually ‘knowing what we are testing’ (see Section 3.0). Improving how we characterise MNMs can help to interpret results of hazard testing and can subsequently confirm the outcomes of risk assessment. It might also help to test such results in different contexts i.e. for other MNMs. Gaps and challenges in characterisation are discussed, as these may have significance for the applicability of policies or legislation as knowledge progresses, and as new nanoforms are developed. Exposure is considered, as a major factor in the risk that nanomaterials pose; different nanoforms also act differently in transport, and there are several reasons discussed why the data on real environmental concentrations is scarce. Hazard is another major factor in assessing risk; the report attempts to present the current understanding of the effects on organisms. The report will have an emphasis on the ecotoxicological risks of nanomaterials, where currently less is known than for risks to human health.

Furthermore, by focussing on methodological considerations of hazard and exposure assessment, this report aims to provide a more enduring and useful picture for risk assessment, which is addressed in another section.

The following sections will be used to structure the rest of the report:

2. State of science of nanomaterials;
3. Characterisation;
4. Exposure;
5. Hazard: toxicology and ecotoxicology;
6. Risk assessment;

These are loosely based on Themes of the MARINA project (Characterisation, Exposure, Hazard, Risk) and the first objective of the NANoREG project objectives.  

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22 For more information see http://www.marina-fp7.eu/activities/
23 Providing legislators with a set of tools for risk assessment and decision making instruments for the short to medium term, by gathering data and performing pilot risk assessment, including exposure monitoring and control, for a selected number of nanomaterials used in products; For more information see http://www.nanoreg.eu/project/nanoreg-objectives
2. State of science of nanomaterials

2.1 History

Nanomaterials have been in existence for some time. For example, they have been used by potters and glassmakers since the Bronze Age; to produce a metallic luster on ceramics since the 9th Century in Islamic areas; and in the vibrant stained glass windows of European cathedrals between 6th-17th centuries C.E.. In the 13th-18th centuries, weapons like the ‘Damascus sabers’ were manufactured, which contained cementite nanowires encapsulated by carbon nanotubes — an ultrahigh carbon steel material that was strong, resilient and held a keen edge (Reibold et al., 2006). However, the manufacture of nanomaterials to fulfil a specific purpose is relatively new.

Nanotechnology was predicted before the practical techniques existed to make it a field of study. Richard Feymann’s lecture in 1959 ‘There’s Plenty of Room at the Bottom’ is often cited as the first time the concept of ‘nano’ was proposed. In the lecture he described a process in which scientists would be able to manipulate and control individual atoms and molecules. However, it was not until 1981, with the development of the scanning tunneling microscope that could ‘see’ individual atoms, that modern nanotechnology really started (National Nanotechnology Initiative [no date]) When scientists have the right tools, they can intentionally make materials at the nanoscale to take advantage of their enhanced properties. As the understanding of nanomaterials and technology improves, there is greater scope to produce purpose-specific nanomaterials by taking advantage of their properties.

Gilbertson et al. (2015) emphasise that more knowledge is needed on the link between the structure and properties of MNMs and their function and also their possible hazards. This knowledge will increasingly allow scientists to manipulate the structure and properties of nanomaterials in order to optimise function and minimise hazard.

2.2 Advances in applications and manufacture

Nanotechnology currently has a multitude of applications in healthcare, energy and environmental remediation and has contributed to the development of new consumer products in areas such as textiles, food and cosmetics. Some examples of potential advances in environmental applications are featured in Box 4.

“There’s plenty of room at the bottom” (Richard Feynman, 1959)
Box 4. Examples of potential nanotechnology applications in the environment

‘Low energy water purification enabled by nanomaterial-coated sponges’ (Liu et al. 2013)

There are a number of treatment methods for disinfecting drinking water but most have high maintenance costs, high energy consumption or negative environmental impacts. One possibility to avoid these disadvantages is a process called ‘electroporation’, in which water is disinfected using an electric field. This kills harmful microorganisms by forming pores in their cell walls. Earlier studies have shown that the process works but needs high voltage electric fields, which use large amounts of energy. However, this study by Liu et al. has shown how the use of nanomaterials could drive down energy use by coating commercially available polyurethane sponges with carbon nanotubes and silver nanowires. This turns the porous structure of the sponges into small conductive electrodes. The researchers tested the efficiency of the ‘nanosponge’ at killing microorganisms and found that nearly all of four diarrhea-causing bacteria were removed from contaminated water when it was passed through the nanosponge. The technology could be useful as a portable decontamination device, as well as for use in larger water treatment systems.

‘Pomegranate-inspired battery design doubles stored energy’ (Liu et al. 2014)

Lithium-ion batteries are the rechargeable batteries used to charge phones, tablets and laptop computers. There is significant interest in making more efficient lithium-ion batteries for commercial as well as sustainability reasons. This new battery design replaces the graphite (solid carbon) anodes of ordinary lithium-ion batteries with anodes made from silicon and carbon nanomaterials. Silicon nanoparticles form the ‘seeds’ of the pomegranates, which are encased in a thin carbon framework. Each silicon seed is housed in its own carbon bubble, with room to rattle around. Practically, the pomegranates exist in powder form, with the pomegranate structure only visible under a microscope. In tests, the new silicon-based anode was capable of storing more than twice as much energy as an ordinary graphite anode. It was also very stable. In a previous study, the researchers showed that the silicon for their battery anodes could be extracted from rice husks, a recycled resource.

‘Making nano-scale manufacturing eco-friendly with silk’ (Kim et al. 2014)

Nanolithography is a way of making finely detailed patterns or structures, such as those found in advanced computer microchips. This is done by coating an underlying base, such as silicon, with a polymer, called a ‘resist’. A design is then ‘drawn’ on the resist, typically using a narrow beam of light or electrons. This changes the properties of the resist and reveals the design by allowing a solvent to either dissolve the drawn area (positive resists) or the area around it (negative resists). Traditionally these solvents are toxic and corrosive chemicals but researchers are now turning to the natural world in search of green solutions, one of which may be silk. Silk proteins have a natural tendency to self-assemble into different structures under different conditions, for example they dissolve in water under some conditions, but are insoluble under other conditions. This suggests it may be possible to produce both positive and negative silk resists. The researchers in this study coated silicon and quartz slides with silk proteins to produce both positive and negative resist types. They then used an electron beam, at different strengths, to draw a design on to the resists. The results demonstrate that silk proteins may be a viable alternative to conventional techniques for nanolithography, avoiding the use of toxic chemicals and the generation of environmentally hazardous toxic waste products.
2.2.1 Bioynthesis of nanomaterials

More recently there have been advances in developing ways to manufacture (mainly metallic) nanomaterials intra- and extra-cellularly by unicellular or multicellular organisms. Indeed, recently, the biosynthesis of nanosized particles, wires, flowers, tubes has been reported successfully (Kuppusamy et al., 2016). Nanomaterials are popularly synthesized by bacteria, fungi and plant extracts; where a so-called ‘bio-template’ of fungi, bacterial or plant extract is used. Not only is this method less harmful to the environment — because of the reduction in use of chemicals — and simpler — with only a single step bioreduction process — but can also be quicker and more cost effective (Singh & Jain 2014; Kuppusamy et al., 2016).

Examples include the use of fungus Neurospora crassa to synthesize gold-silver bimetallic nanoparticles, and the biosynthesis of Zinc Oxide (ZnO) nanoparticles with the bacteria, Aeromonas hydrophila.

Plant extracts have also been used to synthesize nanoparticles. This could have advantages over synthesis using microbes as there is no need for the process of culturing and maintaining fungi or bacteria. Plants also contain abundant natural nutrients such as alkaloids, flavonoids, terpenoids and phenolic acid, which can be extracted from leaves, stems, roots, shoots, flowers, barks, and seeds. Such natural products can act as potential precursors for the non-hazardous synthesis of nanomaterials; these secondary metabolites seem to act as reducing, capping (preventing particles from growing) and stabilising (preventing the particles from agglomerating) agents in the bioreduction reaction that produces the nanoparticles — as well as producing non-hazardous by-products (Kuppusamy et al., 2016). They have also been found to speed up the reduction time, for example, in a bioreduction of silver nanoparticles using horse gram (Macrotyloma uniflorum).

Aroman and Philip (2012) found that fenugreek seed extract has the ability to perform dual functions of reduction and stabilisation of gold NPs. The researchers conclude that one possible reducing agent was the flavonoids, and that the ‘capping’ material responsible for stabilisation was proteins present in the fenugreek extract. The biosynthesised gold nanoparticles showed comparable catalytic activity to gold nanoparticles manufactured in other ways.

Plant extracts have also been used successfully in the more environmentally friendly synthesis of nanoparticles such as cobalt, copper, silver, gold, palladium, platinum, zinc oxide and magnetite (Singh & Jain 2014; Kuppusamy et al. 2014). The biosynthesis reaction can change the size and shape of the nanoparticles, depending on the metal concentration and amount of plant extract – and specific morphological features, such as spheres, can be produced (Gopinath et al., 2012).

Some biosynthesised nanomaterials — such as the silver nanoparticle spheres produced by bioreduction with long pepper (Piper longum) leaf extract (Jacob et al., 2012) — may actually have enhanced cytotoxic effects because of the influence of the plant extract. Silver has been long known for its ability to combat infection, due to its ability to inhibit aspects of respiration; silver nanoparticles have larger surface areas, which increases the area for interactions, making them even more effective anti-microbial agents. Earlier studies had shown that silver nanomaterials can be effective in cancer medicine, and also that black pepper (Piper nigrum) and betel leaf (Piper betel) had cytotoxic and antiproliferative effects on particular cancer cell lines. When silver MNMs were
produced using long pepper extract, they were found to be very efficient in exerting cytotoxic effects, which the researchers say may be due to the presence of alkaloids in the long pepper, which act as capping agents, preventing the particles from growing (Jacob et al., 2012).

There are many studies comparing the effects of biosynthesised nanoparticles with more conventional medical chemicals, or comparing different methods, sizes and concentrations of biosynthesised nanoparticles — for example, biosynthesised gold nanoparticles have been found to be more efficient in combatting liver cancer cell lines than the standard cancer drug cyclophosphamide (Rajeshkumar, 2016). However, the review for this report did not find any studies explicitly comparing the effects of conventionally produced nanoparticles with bionanoparticles, suggesting this may be a useful area of exploration for researchers.

The applications for metallic MNMs are many and varied and these more ‘environmentally friendly’ methods do not remove or mitigate the harmful effects of the nanoparticles once produced. However, some researchers have suggested that other opportunities may arise from nanoparticle biosynthesis. If the proteins involved could be put into a suitable bacterial chassis, for example, then pathways may be engineered to produce more valuable nanomaterials (Edmundson et al., 2014). More generally it has been proposed that the principles of ‘greener’ chemistry could be applied more effectively to nanomaterials to maximise performance and minimise harmful impacts of manufacture (Gilbertson et al., 2015).

2.3 Nanosafety

The field of nanomaterials is moving fast, as new MNMs, new applications for existing MNMs and new methods to produce MNMs develop. However, assessing the hazard associated with nanomaterial exposure, and the characterisation of risks have not kept pace with nanotechnology advances (Klaine et al., 2012; Rio-Echevarria & Rickerby, 2015). It is not always clear whether current test guidelines for assessing nanoparticle hazard are optimal. There is a lack of adequate data to validate the hazard assessment methods that do exist; this may also throw into question some of the exposure limit or hazard data already produced.

This gap exists firstly because the scientific research required to develop nanotechnology does not yield adequate data to also assess the effects (hazards) of those products. Secondly, some of the available scientific data from industry is not adequate for regulatory risk assessment, which requires more robust data sets (Klaine et al., 2012). There is also generally a lack of agreed standardised approaches to characterise the toxicological behaviour of MNMs (Kah & Hofmann, 2015). The regulatory test
methods for assessing hazardous effects are in the process of being confirmed and adapted: an OECD programme is currently reviewing and updating some of the OECD’s test guidelines for nanomaterials. The work is based on the OECD’s 2009 Preliminary Review (OECD 2009), together with outcomes from the OECD WPMN testing programme (Rasmussen et al., 2016) and horizontal expert meetings (OECD, 2012a; 2012b; 2014a; 2014b; 2014c).

There is increasing use of models to estimate the fate and behaviour of MNMs in the environment and their potential environmental impacts, but there is a lack of adequate data to feed into these models. More information is needed on industrial releases, and the percentage and form of MNMs released from final products. This can only be achieved by means of collaboration between industry and research (Kah & Hofmann, 2015).

The rise in the number of articles on nanotoxicology over the past 20 years has been almost exponential. Before the year 2000 only a couple of hundred papers had appeared under the topic of ‘Nanomaterials: environmental and health effects’ but this number has exploded since 2001 and is now above 10 000 (Krug, 2014). However, even as hazard assessment methods are adapted or developed it is a major challenge for the research to ensure that new knowledge is applied and that some of the already identified mistakes are not repeated, and to keep pace with the accelerating development of emerging nanomaterials (see Section 7.2).

One approach that aims to tackle this issue is Safe(r) by Design (SbD). This proposes minimising the hazardous properties of a substance from the very initial stages of its development so any issues related to risk are already addressed in the design phase. SbD is not a new concept, and has been applied to chemicals and chemicals-enabled technologies in general. However, it would be particularly appropriate to apply the approach to MNMs, as they are ever increasing in number on the market (see Section 6.4 for more details on how SbD is being put into practice for MNMs).

2.4 Knowledge gaps and challenges

The challenge of assessing the safety of nanomaterials has long been recognised. In 2006 Andrew Maynard and colleagues laid out five challenges to stimulate research into nanomaterial safety in a commentary paper in Nature (Maynard et al., 2006). These are summarised in Box 5 along with a summary of how far these challenges have been met, indicating the areas where improvements in scientific research are still required.

Although there has been progress in addressing these challenges, there is still room for improvement. In his review of over 10 000 studies on the toxicology of nanomaterials, Krug concluded that the picture for
Box 5. Five grand challenges for research into nanomaterials safety from the 2006 Nature paper (Maynard et al. 2006)

1) Develop instruments to assess exposure to engineered nanomaterials in air and water (within the next 3 -10 years). Maynard and colleagues called for a need to develop smart sensors to monitor airborne nanomaterials. In addition they highlighted the problem of water pollution from the effluent of nanomaterial manufacturing processes, the use of substances containing nanoparticles such as sunscreens and the disposal of products containing nanomaterials. As such they suggested there is a need for research into instruments to track the release, concentration and transformation of MNMs in the water systems.

2) Develop and validate methods to evaluate the toxicity of MNMs (within the next 5-15 years). The Nature commentary paper proposed a need to reach international agreement on a set of screening tests for human and environmental toxicity, which then need to be validated. The authors also suggested that there should be a drive to find alternatives to in vivo testing in animals. Both the OECD and the EU have worked on this since Maynard’s original statements and there is major policy activity to reduce in vivo testing for all chemicals.

3) Develop models for predicting the potential impact of MNMs and the environment and human health (within the next 10 years). Maynard and colleagues highlighted the need to develop validated models to predict the release, transport, transformation, accumulation and uptake of MNMs in the environment. These models should then be used to engineer nanomaterials that are safe by design. This means the suppression of hazardous properties should be included from the very early stages of the development of a new nanomaterial to ensure an unharmful material upon disposal. Since the Nature paper this has been addressed by the NANoREG project (see section 6.4.3) in establishing the concept in nanomaterial innovation and production. However more work is needed to ensure its implementation.

4) Develop robust systems for evaluating the health and environmental impact of MNMs over their entire life (within the next 5 years). The commentary paper suggested a more holistic approach is needed to evaluate the impact of nanomaterials in the form of life-cycle analysis (LCA). This is an issue for all chemicals but at the time of Maynard’s paper there was very little application of this approach to MNMs. The LCA approach will consider impacts of nanomaterials from initial manufacture, through their use to their ultimate disposal. Since the Nature paper more research has used life cycle analysis (see section 6.3). However the analyses themselves still need better data and there is also a need to establish how LCAs and risk assessments can work together to give the most useful and applicable information.

5) Develop strategic programmes that enable relevant risk-focused research within the next 12 months. More mechanisms are needed to enable collaborative and interdisciplinary research programmes between government and industry or between different stakeholders. Alongside this better communication is needed on nanotechnology risks and benefits outside the scientific community. There is a need to build up international information sharing networks between both public and private sectors in the field of nanotechnology safety research. This is now a well recognised need and since Maynard’s publication several European Commission projects with the Nanosafety cluster (see box 1.4) have incorporated this into their work.
nanotoxicology was not much clearer than ten years previously and that several important aspects had still not been investigated (Krug, 2014). More specifically, he concluded that there was a large diversity in the methods used in the studies, which meant it was difficult to make comparisons between them, and which could also be responsible for the sometimes contradictory findings. He also pointed out that the majority of studies did not consider it necessary to characterise or describe the properties of the MNMs (see Section 3) and that this reduces their significance. He called for an international agreement on the need to include characterisation in all studies on health hazards of MNMs.

On this basis of his review he made the following recommendations:

- The standard protocols and methodical processes established during support programmes must form an integral part of new incentive projects.
- The development of appropriate and inexpensive analytical methods should be integral to all funding programs.
- The links between in vitro and in vivo experiments must be improved, as must extrapolations based on in vitro experiments.
- The gaps in scientific knowledge (e.g. regarding certain exposure pathways such as the gastrointestinal tract) should be specifically targeted in new research programmes.
- Long-term studies on the possible accumulation of nanomaterials should be integrated into future incentive measures and support programmes.
- The comparability of studies must be achieved by the integration of toxicological expertise into all projects.

The OECD has also produced a revised version of their Guidelines on sample preparation and dosimetry, which is a set of outlines intended to help laboratories to apply the current OECD Test Guidelines to determine the physical, chemical and toxicological properties of water-insoluble nanomaterials. However, it must be noted that this document focuses on the kinds of tests that address endpoints and types of nanomaterials being tested by sponsors of the Working Party on Manufactured Nanomaterials programme.

2.5 Current research strategies on nanosafety

The European Commission has been acting on the identification of these challenges and knowledge gaps by funding a large number of research projects through FP6 (2002-2006), FP7 (2007-2013) and Horizon 2020 (2014-2020). These fifty or so projects have a central forum called the European Nanosafety Cluster, which aims to maximise the synergies between the projects. The European Nanosafety Cluster produced a research strategy for the European Commission (Savolainen et al. 2013).

The research strategy calls for an urgent need to develop not necessarily a ‘nanotoxicology’ but rather a ‘new’ toxicology for the 21st century. It cites a 2007 report by the US National Academy of Sciences on behalf of the US Environmental Protection Agency (EPA) that proposes a new ‘systems toxicology’ approach — i.e. addressing toxicology questions with some of the computational methods of systems biology. The overall aim is to promote a shift from testing toxicity primarily in animal models to testing in vitro and in vivo using lower model organisms such as worms, flies and zebrafish. This would go alongside computational modelling, enabling toxicology to develop from an observational science into a predictive science. The report proposes that this is a much-needed development with the accelerated growth of so many new nanoforms, which need assessment before they start start being mass produced.

The research strategy also identifies four major research areas, which are described in Box 6. These are also the areas into which this In-depth Report is organised.

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24 *In vitro* experiments are those conducted in a controlled environment outside of a living organism, for example in petri dishes, test tubes etc. They are mainly conducted at the level of cellular biology. *In vivo* experiments are those conducted within a whole, living organism.
Box 6. Research strategy on Nanosafety in Europe 2015-2025 (Savolainen et al. 2013)

1) Material identification and classification. As discussed previously, most definitions of a nanomaterial concentrate on size. Size is a sensible property to decide if a material is nano, but for actual risk assessment there is a need to consider further properties. Approaches to classifying materials then need to consider which characteristics are associated with impacts.

2) Exposure and transformation. Research has shown that the properties of MNMs can change during their life cycle or after their release into the environment. Possible areas and processes where this could occur such as production, use, aging and end-of-life were highlighted. Research is needed to understand what transformations occur during these processes and how this influences the exposure to the environment.

3) Hazard mechanisms including both human toxicology and ecotoxicology. The research strategy reports that more research is needed to understand the mechanisms which lead to toxicity and to identify those humans and aspects of the environment that are susceptible to toxicity. In addition there is a need to better identify the main properties that influence the interactions with biological systems. Focussing on the environment, research is needed to establish the life cycles of MNMs, to improve the prediction of the (bio)degradation of organic nanomaterials and to develop standardised test methods for water environments and soil.

4) Risk prediction tools. Traditional risk assessment frameworks follow the four-step paradigm: 1) hazard identification; 2) hazard assessment; 3) exposure assessment; and 4) risk assessment. The research priorities on the risk management include improving models for exposure to nanomaterials, better studies on environmental impact and better integration of life-cycle assessment (LCA) into risk assessment. There is also a need for research into the integration of safe-by-design and green nanotechnology approaches into development stages of new nanomaterials and their applications.

More recently the NANoREG project has also looked at how nanomaterial safety research outcomes need to be further validated for regulatory purposes. The project conducted a scientific analysis to answer a set of regulatory questions about the safe use of nanomaterials (Dekkers, Sips and Cassee, 2015). This concluded that most data gaps are related to:

- characteristics (e.g. size, surface area, surface chemistry, solubility, shape) that influence the risk of nanomaterials to the environment and to humans;
- standardised methods to determine these characteristics; and
- nano-specific risk assessment strategies and approaches.

The NANoREG report has identified a series of both short-term and long-term research needs, which it has organised according to a set of regulatory questions which are summarised in Table 2.
Table 2: NANoREG Regulatory data gaps and research needs (Dekkers, S., Sips, A.J.A.M. and Cassee 2015)

<table>
<thead>
<tr>
<th>Regulatory question</th>
<th>Data gaps and research needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>How can nanomaterials be identified for the purposes of risk assessment, as well as according to the EU definition of nanomaterials?</td>
<td>• In the short term research is needed to develop reasonably priced, accessible, standardised and validated methods and procedures to quickly identify and quantify nanomaterials according to the EC definition. In the long term, researchers and funders should try to further clarify and understand the key characteristics that influence the safety of nanomaterials.</td>
</tr>
<tr>
<td>How do nanomaterials change over time and use?</td>
<td>• In the short term more research is needed to understand how nanomaterials change over time, for example when they are burnt or dissolved. Long-term research is needed to further standardise and validate methods to test or predict the extent to which nanomaterials change and the rates at which this happens.</td>
</tr>
<tr>
<td>Can risk assessment information from different forms of nanomaterials (or from the bulk material) be ‘re-used’?</td>
<td>• In the short term researchers need to develop scientific approaches to apply existing information about the risk of well-known nanomaterials and bulk materials to new nanomaterials. Methods such as extrapolation, interpolation, read-across and grouping could help quantify the most important characteristics that influence the risks of nanomaterials (see section 5.7.2). Long-term research is needed to further develop and to implement these approaches: such approaches inherently entail some uncertainty and it is important to know how much uncertainty is acceptable from a regulatory perspective. Furthermore, quick tests to assign nanomaterials to the right group (with grouping based on knowledge of properties/characteristics that influence nanomaterial risks) or to justify read-across, extrapolation, or interpolation should be developed.</td>
</tr>
<tr>
<td>Will nanomaterials accumulate in man, the environment and environmental species and what are the driving forces?</td>
<td>• Short-term research is needed to fill the data gaps on accumulation of nanomaterials. In the long term, research is needed to identify and verify the most important characteristics and properties of nanomaterials that influence accumulation.</td>
</tr>
<tr>
<td>What are the critical characteristics of nanomaterials that need to be considered to develop safer nanomaterials?</td>
<td>• In the short term there is a need to utilize the current knowledge on important characteristics that influence risk in the design of new nanomaterials. Long-term remaining research needs include further developing and implementing the approaches for safe-by-design (see section 5.4.3).</td>
</tr>
</tbody>
</table>

As developments in the field of nanomaterials continue and research papers proliferate we can only hope to get a snapshot of the situation. Evident knowledge gaps exist but as we close them down, others are likely to open as MNMs continue to change and develop.

Research into the risks of nanotechnology must continue alongside development of nanotechnology itself to inform future policy and maintain European industrial competitiveness. This requires the cooperation of researchers from many different fields, as well as collaboration between industry and academia.

Having summarised the state of scientific research into nanomaterials the following sections will consider current scientific messages in the field of nanosafety in terms of characterisation, exposure, hazard and risk assessment.
3. Characterisation

As the production of different MNMs grows, clarity and rigour are needed in describing nanomaterials in terms of their physical and chemical properties so that they can be effectively differentiated.

There is increasing evidence to suggest that minor differences in physicochemical properties can influence the transport and distribution within environmental compartments as well as potential adverse effects of a nanomaterial (Pettitt & Lead, 2013). Thus an effective characterisation of MNMs is needed to identify relevant physicochemical differences, in order to assess hazard and risk.

Nanometrology is an area of study concerned with measurements at the nanoscale and is therefore important to characterisation. Some of the most common measurement techniques used in nanometrology are atomic force microscopy, electron microscopy and X-ray diffraction, giving information on some, but not all, of the relevant properties, e.g. size and size distribution, 3D shape, crystallinity, surface topography and hardness, to give some examples (Kuznetsov et al., 2015; Geiser et al., 2005; Fregnaux et al., 2013). However, there is a lack of standardised methods and sampling strategies, which does slow down measurements of exposure, for example.

3.1 Existing methods for characterisation

In order to adequately characterise MNMs for safety assessments, there needs to be agreement on the set of properties used to describe MNMs and the techniques used to measure these properties.

Scientific research can help develop a set of descriptors that facilitate accurate material characterisation and which are likely to predict how the MNM may behave physically, chemically and maybe toxicologically, both in the environment and in organisms.

3.1.1 Physicochemical properties

There are a large number of physicochemical properties that could potentially be considered in characterisation. Researchers and scientific bodies have therefore aimed to list a minimum set of properties. Below is a summary of properties that commonly feature in these proposed minimum sets. Table 3.1 then describes the specific proposals on the properties that need to be considered in more detail. The proposals come from five different sources: SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks (2009), Pettitt & Lead (2013), OECD-WPMN (2016), Guide & Work (2015), and Rausher et al. (2015).

**Size**

This refers not only to the physical dimensions of a particle but, for collections of particles, the distribution of the sizes of the particles (Guide & Work, 2015). **Size distribution** is important; considering the relative number of particles in a material takes account of the fact that not all particles in a material will be completely uniform in size.

One consequence of using a number-based size distribution is that sometimes materials classified as nanomaterials (e.g. more than 50% of constituent particles by amount) still have a very low mass fraction of nanoparticles (Rauscher et al., 2015).
Also, nanoparticles may exist either as single particles or as constituent particles of agglomerates or aggregates which are larger than individual particles. In many cases, nanoscale properties will not be retained when aggregated — however, some agglomerates or aggregates with external dimensions larger than 100 nm do retain some nanoscale properties (Rauscher et al., 2015).

When describing a nanomaterial it is therefore important to indicate both the mean particle size and the distribution of size. Both the size distribution of constituent (primary) particles, as well as the size distribution of particles as they present themselves in the media of interest (which will often/mostly include aggregates and agglomerates of primary particles) are required. (SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) 2009).

For characterisation, it is important to report clearly which metric is employed in the description of size distribution (e.g. by mass or by number) and how the potential presence of aggregates and/or agglomerates is treated (which may, in addition, be highly dependent on the media and any pre-treatment of the sample).

Shape

Shape is an important area for our understanding of nanomaterials. They can take a wide variety of shapes, including rods, spheres, fibres, stars, ferns, flowers, pomegranates, irregular and random shapes and many more, which may lead to differences in properties. In general the shape of MNMs has been shown to influence their uptake by cells (Pettitt & Lead, 2013) and therefore their potential adverse effects. In particular, nanoparticles with a high aspect ratio or long fibre-like shape have attracted attention from toxicologists because of their potential ‘asbestos-like’ behaviour in the lungs.

Surface Properties:

The specific surface area is often defined as the surface area of a substance divided by its volume (Guide & Work, 2015), or VSSA (volume-specific surface area). Toxicological data suggest that the total surface area of nanoparticles is an important metric in describing toxicological responses in biological systems (Pettitt & Lead, 2013). This is because the surface is where many reactions occur so, in general, the greater the area of the surface, the more ‘reactive’ the substance is. Particle shape and aggregation, as well as dispersity (the measure of distribution of molecular mass in a material) could reduce the VSSA for a particular particle size (Rauscher et al., 2015). VSSA would not suffice as a defining metric of nanomaterials, since particles with a large size but a very high surface area (because of the presence of pores or coatings) would then fall into the classification of nanomaterials.

Manipulating the surface chemistry of nanoparticles is becoming increasingly sophisticated and often takes the form of coatings. The modification can influence agglomeration behaviour and the level to which the materials dissolve and disperse in water environments, transportation and ecotoxicity. As such it is likely that MNMs with the same core chemistry, but different surface modifications may behave differently in the environment so this property is important in assessing hazard and risk (Pettitt & Lead, 2013).

In an environmental context the surface charge a particle carries will influence its aggregation in water and its mobility and transport through surface waters and porous media (Pettitt & Lead, 2013). As such it is another important surface property to consider when assessing risk.

Composition and Structure

This is the chemical information of the nanomaterial (Guide & Work, 2015) and should include the degree of purity, as well as any known impurities or additives (SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), 2009). Whether a MNM is in a crystal form or not also alters its toxicity (Pettitt & Lead, 2013) so is an important structural property to consider.

The SCENIHR put forward a list of physicochemical properties required to adequately describe nanomaterials in its opinion on Risk Assessment of Products of Nanotechnologies (Scientific Committee
on Emerging and Newly Identified Health Risks, 2009). In their paper on characterisation requirements for nanomaterial regulation Pettitt and Lead propose a minimum list of physicochemical properties required to characterise NMs for regulatory purposes (Pettitt & Lead, 2013). In project MARINA’s Good Practice Guide for assessment of nanomaterials, Guide and Work list the physicochemical properties proposed by ISO (International Standardisation Organisation) and OECD (Organisation for Economic Cooperation and Development) (Guide & Work, 2015). The OECD Working Party on Nanomaterials (WPMN) have recently updated the list of properties in their report evaluating the methods applied in the OECD-WPMN testing programme(OECD-WPMN, 2016). The proposed properties from these sources are listed in Table 2.

### Table 3: Comparative list of nanomaterial properties from various sources

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Properties taken into account</td>
<td></td>
<td>Size</td>
<td>Size</td>
<td>Size distribution</td>
<td>Size distribution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Particle size distribution</td>
<td>Particle size distribution</td>
<td>Particle size distribution</td>
<td>Size distribution</td>
</tr>
<tr>
<td>Agglomerate morphology</td>
<td></td>
<td>Aggregation/ agglomeration</td>
<td>Aggregation/ agglomeration</td>
<td>Aggregation/ agglomeration state</td>
<td></td>
</tr>
<tr>
<td>Mass particle number</td>
<td></td>
<td>Shape</td>
<td></td>
<td>Shape</td>
<td>Aspect ratio</td>
</tr>
<tr>
<td>Surface area</td>
<td></td>
<td>Specific surface area</td>
<td>Specific surface area</td>
<td>Specific surface area</td>
<td>Specific surface area</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Surface morphology/ topography</td>
<td></td>
</tr>
<tr>
<td>Crystallite form</td>
<td></td>
<td>Crystallite size</td>
<td>Crystallite phase</td>
<td>Crystallite size</td>
<td>Structure, including crystallinity and defect structure</td>
</tr>
<tr>
<td>Composition</td>
<td></td>
<td>Chemical composition</td>
<td>Composition of nanomaterial (including degree of purity, known impurities or additives)</td>
<td>Structural formula/ molecular structure</td>
<td></td>
</tr>
</tbody>
</table>
### 3.1.2 Techniques to analyse physicochemical properties

There is a large number of techniques to characterise MNMs and these vary according to the property. The REACH-CLP-Biozid Helpdesk at the German Federal Institute for Occupational Safety and Health has produced a report to provide advice to those planning to register nanomaterials with REACH. This provides a useful list of analytical methods to characterize MNMs, which is shown in Table 4.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell chemistry/ coating/ Surface modification</td>
<td>Surface chemistry</td>
<td>Surface chemistry</td>
<td>Surface chemistry</td>
<td>Surface chemistry (composition, charge, tension, reactive sites, physical structure, photocatalytic properties, zeta potential)</td>
<td>Phase identity</td>
</tr>
<tr>
<td>Surface charge</td>
<td>Surface charge</td>
<td>Surface charge</td>
<td>Zeta potential</td>
<td>Hydrophilicity/ lipophilicity</td>
<td></td>
</tr>
<tr>
<td>Solubility/ dispersibility</td>
<td>Solubility/ dispersibility</td>
<td>Water solubility/ dispersibility</td>
<td>Dustiness</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sources: Pettitt and Lead (2013); ISO/TR 13014: 2012; OECD (2012); OECD (2016); SCENIHR (2009)

All these analytical techniques have their specific reliability and sensitivity profiles and typically need to be combined to obtain reliable and individual assessments (SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), 2009). In order to apply these techniques to a high level of performance, a number of issues need to be considered for each case, for example accuracy, specimen preparation and presence of contamination.
Pettitt & Lead (2013) strongly advocate using multiple methods where possible to describe a physicochemical property (Pettitt & Lead, 2013). Specifically Goenaga-Infante & Larsen (2014) suggest that the combination of field-flow fractionation with elemental and sizing detectors has emerged over the last decade as a highly promising approach for size-based detection of nanomaterials in complex samples (Goenaga-Infante & Larsen, 2014). Using multiple methods however, requires a vast array of analytical equipment, man-hours and expertise in sample preparation and data analysis. Availability of these equipment and expertise is often therefore restricted to large centres and there needs to be an appropriate strategy to facilitate access to such resources. For example, one solution is to establish national accredited facilities for nanomaterial characterisation, perhaps building on existing centres of excellence, as has happened in the research community in the UK and EU (Pettitt & Lead, 2013).

Table 4: List of suitable analytical methods for the characterisation of nanomaterials (OECD-WPMN 2016)

<table>
<thead>
<tr>
<th>Source</th>
<th>Pettitt &amp; Lead (2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characterisation</td>
<td>Analytical method suitable for broad range of nanomaterials in general</td>
</tr>
<tr>
<td>Chemical composition</td>
<td>X-ray photoelectron spectroscopy (XPS)</td>
</tr>
<tr>
<td>Aggregation and agglomeration</td>
<td>Atomic Force Microscopy (AFM)</td>
</tr>
<tr>
<td>Particle size distribution</td>
<td>Centrifugal Liquid Sedimentation (CLS) Dynamic Light Scattering (DLS)</td>
</tr>
<tr>
<td></td>
<td>Differential Mobility Analysis (DMA) Transmission Electron Microscopy (TEM)</td>
</tr>
<tr>
<td></td>
<td>Scanning electron microscopy (SEM)</td>
</tr>
<tr>
<td>Crystalline phase</td>
<td>X-Ray Diffraction (XRD)</td>
</tr>
<tr>
<td>Dustiness</td>
<td>Rotating drum Small rotating drum</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>Brunauer-Emmett-Teller-Method (BET)</td>
</tr>
<tr>
<td>Water solubility/dispersibility</td>
<td>Shake flask method</td>
</tr>
<tr>
<td>Zeta potential</td>
<td>Electrophoretic light scattering (ELS)</td>
</tr>
<tr>
<td>Photocatalytic activity</td>
<td>Different methods suitable for different types of MNMs</td>
</tr>
<tr>
<td>Radical formation potential</td>
<td>Electron paramagnetic resonance (EPR) / electron spin resonance (ESR)</td>
</tr>
<tr>
<td>Crystallite size</td>
<td>X-ray diffraction (XRD)</td>
</tr>
<tr>
<td>Surface chemistry</td>
<td>Different methods suitable for different types of MNMs</td>
</tr>
<tr>
<td>size</td>
<td>raction</td>
</tr>
</tbody>
</table>
3.2 Knowledge gaps and challenges

Measuring the physicochemical properties of nanomaterials is not as straightforward as it is for bulk chemicals. As previously discussed, nanomaterials are not a homogeneous group and there can be different forms of the same nanomaterial which means obtaining reliable data is not easy. Often, this leads to a situation in which experimental data gets reported without proper understanding of the associated errors and the impact of these errors on the final result. Sources of errors may arise from a number of factors including: a high ‘polydispersity’ of the nanomaterial sample (i.e. a heterogeneous sample, comprising differences in size, shape and distribution of mass as discussed in Section 3.1), the influence of biological environment on the nanomaterial and the fitness for purpose of analytical methods used (Guide & Work, 2015). There is recognition that studies and reports on the properties and behaviors of nanomaterials often have inadequate or incomplete characterization. As a consequence, the true value of the data in these reports is, at best, uncertain (Baer et al., 2013).

3.2.1 NMs as dynamic systems

The biological environment in which the particles are dispersed can pose challenges where measurement is concerned. Nanomaterials interact dynamically with the media in which they exist so particles can change shape, agglomerate and/or sediment out, change their chemistry and form protein coatings or coronas (Baer et al., 2013). Therefore the environment into which MNMs are released will mediate the ultimate environmental fate and influence the possible harmful effect of MNMs (Pettitt & Lead, 2013). Due to the inherent challenges this provides, many researchers in the past have often reported the physicochemical properties of the “pristine” particles in the absence of the biological environment.

However, there is now increasing recognition that characterisation should be undertaken in situ to provide a realistic picture, or under comparable experimental conditions. In addition it should be conducted at the beginning and end of any test of ecotoxicity.

3.2.2 Preparation and analysis challenges

Nanomaterials present some unique difficulties in preparation and analysis (Baer et al., 2013). Since MNMs are so influenced by their surroundings in the analysis itself, a critical challenge is the extraction of nano-objects from the sample into a suspension without causing particle transformation. Another major challenge is preserving the stability of nano-objects in suspensions during analysis. Temperature changes, exposure to light and/or increase of sample turbulence may also affect the property of nanomaterials. (Goenaga-Infante & Larsen, 2014).
3.2.3 Multiplicity of methods

There is no single method which can be used for every type of nanomaterial. Based on shape and/or surface properties the set of analytical methods will have to be chosen on a case-by-case basis. Moreover, different methods can be used to describe the same nanomaterial. Therefore, it is important not only to present a measured value, but also to give detailed information on the applied method, the sample preparation and any adaptations which became necessary to conduct the test (REACH-CLP-Biozid Helpdesk, 2012).

3.2.4 Standardisation of methods

In the OECD-WPMN report on Physical-Chemical Properties of Nanomaterials and Evaluation of Methods, expert opinions were gathered on the different techniques. The conclusion was that suitable and widely accepted methods are available for a broad range of nanomaterials to measure several properties: chemical composition, aggregation and agglomeration, particle size distribution, crystalline phase, dustiness, water solubility, zeta potential (the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion), porosity, radical formation potential and size of individual crystals (crystallites). However, most of these methods are not standardised or not standardised for MNMs i.e. there is no set of standards or commonly used guidelines on how to apply these methods. For other properties such as redox potential, experts indicated that all evaluated methods were unsuitable for nanomaterials. In its conclusions the report also highlighted the importance of including a detailed and complete description of the methodology used when reporting on the physicochemical properties of MNMs.

3.2.5 Access to and application of analytic tools

Due to the multidisciplinary nature of the field, not every research team will have access to the range of characterisation tools needed to obtain potentially important information. In addition the range of information needed to understand nanomaterials may require the application of tools and data analysis beyond the expertise of the research teams, sometimes leading to less-than-optimum application of important methods and/or incomplete understanding of the data produced (Baer et al., 2013). As previously proposed, one possible solution may be to establish national centres for characterisation, which houes the necessary tools and are available to researchers.
3.3 Current and future developments

The project MARINA has highlighted the importance of nanometrology or the scientific measurement of nanomaterials.

A major objective of the project MARINA was to extend the range of tools available to researchers. State of the art methods were developed and evaluated, with the aim of allowing measurements under challenging conditions e.g. when particles are in their biological or environmental media.

The recommendations in its report on evaluation of methods to measure physicochemical properties, the OECD-WPMN suggested that to develop and validate standard test methods, there is a need for reference materials. The report also advises that there is a need to define standardised substances or environments in which MNMs tend to exist.

One of the work packages for Project MARINA provides high quality and relevant Representative Nanomaterials (RNM) for measurement purposes for the project. Fourteen principal RNMs were selected and characterised on the basis of the 14 properties over time and at different temperatures. The project has also developed guidance on sample preparation and the RNMS have been extensively tested in vitro and in vivo for ecotoxicology within the other MARINA work packages.

Alongside this the NanoDefine project aims to address the challenge in developing methods that reliably identify, characterize and quantify nanomaterials. It focuses on the implementation of the EU definition so only considers characteristics of size and size distribution. It will explicitly support the challenges associated with the implementation of legislation in this area by:

- addressing the issues on the availability of suitable measuring techniques, reference materials and validated methods that are acceptable for all stakeholders;
- providing an integrated and interdisciplinary approach with a close international cooperation and networking between academia, concerned industries and standardisation bodies.

Fig 2, opposite page: Sources of manufactured nanomaterials (MNMs) to the environment, organised by product group (as defined by Ricardo Energy & Environment, 2016), with example products and MNMs provided for each group.

25 Agglomeration/Aggregation; Water Solubility/Dispersibility; Crystalline phase; Crystallite size; Representative Electron Microscopy; Particle size distribution; Specific surface area; Zeta potential; Surface chemistry; Photocatalytic activity; Redox potential; Radical formation potential; Elemental analysis and Storage stability.
4. Exposure

With the increase in manufacture and use of products that contain nanomaterials, there is an increased likelihood that humans and other organisms will come into contact with MNMs. The level at which this contact occurs is known as the exposure.

4.1 Release of manufactured nanomaterials (MNM)

Release into the environment can happen both intentionally (for example for environmental remediation, disposal after use) or unintentionally (for example through wear and tear of products containing MNMs and accidents). This release occurs throughout the lifecycle of the product i.e. during the production process, the use and disposal (Nowack & Bucheli, 2007; Nowack, 2009; Nowack, 2010).

During the production process, release can occur from accidental spills or when the nanomaterials are transported. When the product is in use and nanoparticles are embedded in a material, they can be released by mechanical, thermal or chemical processes. Examples of mechanical processes are drilling, grinding, sawing, whilst combustion is a typical example of a thermal process. An example of a chemical process is the application of corrosive chemicals on surfaces. Fig 2 provides a schematic diagram of the intentional modes of release to the environment.
There are also mixed processes such as sanding which involves mechanical and thermal processes (Roberts & Fort 2014). Lastly, release can occur during disposal via discharges from wastewater treatment plants, landfills and waste incineration plants, all of which are likely to receive MNM from nano-enabled products when these are disposed at their end-of-life phase. Table 5 provides examples of how specific nanomaterials are released into the environment.

<table>
<thead>
<tr>
<th>Product</th>
<th>Examples of nanomaterials</th>
<th>Potential release and exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosmetics</td>
<td>TiO2 or ZnO</td>
<td>Directly applied to skin, washing off to the environment and the disposal of containers</td>
</tr>
<tr>
<td>Fuel additives</td>
<td>Cerium oxide</td>
<td>Exhaust emissions</td>
</tr>
<tr>
<td>Paints and coatings</td>
<td>Silver nanoparticle coatings and hydrophobic nanocoatings</td>
<td>Wear and washing</td>
</tr>
<tr>
<td>Clothing</td>
<td>Silver nanoparticle coatings and hydrophobic nanocoatings</td>
<td>Absorption by the skin</td>
</tr>
<tr>
<td>Electronics</td>
<td>Carbon nanotubes</td>
<td>E-waste will lead to emission of CNT</td>
</tr>
<tr>
<td>Toys and utensils</td>
<td>Carbon nanotubes</td>
<td>E-waste will lead to emission of CNT</td>
</tr>
<tr>
<td>Combustion processes</td>
<td>Ultrafine particles</td>
<td>Emission with the exhaust</td>
</tr>
<tr>
<td>Soil regeneration</td>
<td>Calcium and phosphorus based nanoparticles</td>
<td>High local emission and exposure</td>
</tr>
<tr>
<td>Production of</td>
<td>Electrophoretic light scattering (ELS)</td>
<td>Byproducts could be emitted locally</td>
</tr>
<tr>
<td>nanomaterials</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


4.2 Emission and transportation of MNMs

Once released, nanoparticles can enter the air, water and the soil, a process known as immission. Normally the nanoparticles enter the environment linked to other particles as agglomerates or aggregates or embedded in the matrix material (e.g. fragments of plastics). They can then be transported or transmitted within the environment. During this transportation process the particles often undergo changes such as decomposition, dilution or changes of the surface. This can significantly alter their characteristics e.g. size, shape, charge, state of agglomeration etc. Nanoparticles tend to agglomerate (coagulate), aggregate (fuse) or a combination of both (Miseljic & Olsen 2014). This change in characteristics can produce nanoforms of the same materials which behave differently (see Section 3).
4.3 Environmental exposure to MNMs

Because of the large amount of factors that can alter nanoparticles, people and the environment can receive very different exposures to the same nanomaterials. Therefore, our knowledge of transformation and current exposure models for conventional substances are not likely to be appropriate for the prediction of exposure throughout the different stages of their life cycle (Savolainen et al., 2013).

Environmental exposure to MNMs represents potentially the most widespread exposure path, and is relevant for the whole population as well as animals and plants. Plants generally come into contact with nanoparticles through their roots via soil or through their leaves via the air. There are also many microorganisms such as bacteria or fungi that are exposed to nanoparticles through soil, air and water (see Figure 3).

Fig 3: Flowchart of the environmental distribution of nanomaterials (adapted from http://nanopartikel.info/en/nanoinfo/basics/987-exposure-basics)
4.4 Existing techniques for measurement

The knowledge on environmental release and exposure is limited (Roberts & Fort, 2014; Gottschalk et al., 2015). Although more research has been done on exposure in the work place, far less research has been conducted on environmental exposure to MNMs after their incorporation into products. Research into release and exposure is further confounded by the large number of contributing factors and there is also a difficulty in terms of acquiring data on patterns of usage of products (Savolainen et al., 2013).

4.4.1 Measuring environmental concentrations

Compared to their bulk counterparts, measuring the environmental concentration of MNMs is a challenge. It is very difficult to put in place the technology to monitor the environmental concentrations of these tiny particles.

The quantification of MNMs in environmental samples, especially in solid samples such as soil and sediment, is still not possible. This is because, on the one hand, MNMs are in most cases not directly detectable by analytic methods due to their very low concentration in the environment. On the other hand, even if detected, there are still major difficulties to differentiate the naturally occurring nanomaterials from the MNMs. In addition the instruments required are expensive and are not yet able to measure accurately anywhere in the environment. Some filtration, microscopic, spectroscopic, chromatographic and other techniques have been combined to detect and quantify the MNMs under laboratory conditions but these are not yet applicable for real time sampling under natural conditions (Gottschalk et al., 2013).

In their review on modelling and measuring studies of MNM concentrations Gottschalk et al. reported that the number of analytic studies that attempted to measure MNMs was sparse (Gottschalk et al., 2013). Examples they provided mainly took samples from wastewater or surface water and used techniques such as electron microscopy and liquid chromatography to measure concentrations. Fewer measured atmospheric concentrations using filters to collect the MNMs in the air.

Fig 4: Scanning electron micrograph of graphene oxide nanoparticles attached to the surface of Artemia salina (microscopy by M, Hočevar IMT Ljubljana, Slovenia; sample preparation by S. Novak UL, BF Ljubljana, Slovenia).
Another, more feasible way to obtain information on levels of MNMs in the environment is to model predicted environmental concentrations (PEC). If these models are robust and have sound input data they can provide a useful estimate on MNM concentrations.

### 4.4.2 Predictive models for environmental concentrations.

There are an increasing number of modelling studies to predict the environmental concentration of MNMs. These efforts combine analytical techniques to provide quantitative information on their occurrence in both the workplace and the wider environment. The major challenge for such models is the enormous variation and uncertainty in the available data about the influential factors. Such high variability and uncertainty were approached and quantified for the first time in 2009 with some probabilistic/stochastic approaches shedding some light on the environmental concentration spectrum that can be expected (Gottschalk et al., 2009). For example, information on the volumes in which nanomaterials are produced, applied and released into the environment. In addition there is a lack of knowledge about the properties that influence exposure (e.g. distribution of size and agglomeration etc.), background concentrations of MNMs already existing in the environment and how nanomaterials behave in the environment in terms of how they disperse, degrade and transform.

Gottschalk and colleagues (Gottschalk et al., 2013) conducted the first review of studies that used models to predict environmental concentrations of MNMs and then compared results to studies that measured environmental concentrations. The goal was to present the methods used and, by comparing predicted and measured concentrations, to evaluate the reliability of the results of the models.

Models vary a great deal. Not only in the MNMs they choose to study but also in terms of the environmental compartment (e.g. surface water, soil and air), the geographical level (e.g. river basin, city or country), source of release (e.g. sewage plants, incineration plants) and of course the modelling methods used (Gottschalk et al., 2015). Box 4.1 contains some examples of modelling studies which provides an indication of the variation.

More recently it has been proposed that dynamic modelling may be more relevant to estimating nanomaterial exposure (Sun et al., 2016). As its name suggests the dynamic model provides estimates over time. This is unlike ‘static’ models, which tend to provide an average estimation (for example, per year, so do not consider variations within that year). This can be a problem for materials that have annual variations, for example nano-TiO₂, which is used in sunscreen and has greater usage during summer. To deal with these variations, the early ‘static’ models were based on conservative risk estimations that assumed that the entire lifecycle emissions would be discharged to the environment over the course of one year, with the aim of preventing any underestimation of the annual release.
Box 7. Examples of modelling studies on environmental concentrations of MNMs

Nanomaterials during waste incineration and landfilling in Switzerland (Mueller et al., 2012)

A study by Mueller and colleagues aimed to model the flows of MNMs during waste incineration and landfilling. They modelled four substances: nano-Titanium Oxide (TiO$_2$), nano-Zinc Oxide (ZnO), nano-silver (Ag) and carbon nanotubes (CNTs). The modelling was performed for Switzerland where almost 100% of the municipal waste and sewage sludge is burned. Despite several differences between the models for the different MNMs most of the MNMs go from the waste incineration plant to the landfill as bottom ash. A different MNM distribution was found for CNTs that are expected to burn to a large extent (94%) so that only insignificant amounts remain in the system. The results of the modelling show that waste incineration can have a strong influence on some MNMs but that the majority of the MNM-mass is still expected to end up in landfills.

Sunscreen TiO$_2$ in UK rivers (Johnson et al., 2011)

In the UK, Johnson and colleagues estimated the concentrations of sunscreen TiO$_2$. They based their modelling on their own measured concentrations in biosolids. This is the organic matter recycled from sewage, especially for use in agriculture. Those measured values were then linked to the proportion of Ti present as nano-TiO$_2$ that was estimated from market information and data on the recommended sludge applications to soils. Concentrations in surface waters in Southern England were then modeled based on commercial information on the use of Ti sunscreen products by the relevant population and data on removal rates for sewage treatment. The highest predicted value was 8.8 µg/L for the Thames region in which it was assumed one in four people used the recommended application of sunscreen. Ecotoxicological studies using potentially vulnerable species indicated that 1,000 µg/L TiO$_2$ MNMs did not affect the viability of a mixed community of river bacteria, even in the presence of UV light.

Concentrations of nine MNMs in Danish environment (Gottschalk et al., 2015)

This research modelled environmental concentrations of nine MNMs in Denmark and was the first to include a number of important methodological aspects. Firstly it distinguishes between photostable TiO$_2$ (as used in sunscreens) and photocatalytic TiO$_2$ (as used in self-cleaning surfaces). Secondly it is the first to present results for exposure in marine water and sediment. Lastly, it provides the first environmental concentrations for quantum dots, carbon black and Copper Carbonate (CuCO$_3$), which is used in wood coatings. Other MNMs that are analysed are ZnO, Ag, CNT and Cerium Oxide (CeO$_2$). The modelling is based on probability distributions of production, use, environmental release and transfer between different environmental compartments such as soil and surface water and technological compartments such as waste-water treatment plants, factories etc. It considered the complete life cycle of products from manufacture to disposal.

The results reveal that in aquatic and terrestrial systems the highest concentrations are expected for carbon black and photostable TiO$_2$, followed by CuCO$_3$ in soils (assuming that the use as wood preservative becomes important). This is on the one hand caused by relatively high production amounts for these materials, but on the other hand by their use in products with significant release. For example carbon black is used in tyres and there is significant release of this because of the wear and tear of tyres from their use on vehicles. In addition these materials do not tend to undergo transformational reactions so their concentrations remain high. On the other hand ZnO and Ag do undergo transformation during water treatments and this results in extremely low concentrations in the environment. For both materials it has
been shown that wastewater treatment can efficiently remove them and also transforms them into the respective sulfide forms.

Transformation reactions are also likely to be important for nano-CuCO$_3$ because it is relatively soluble. However, due to the lack of data for this material the model did not take into account any transformations, meaning the nano-CuCO$_3$ concentrations are likely to be an overestimate. In sludge-treated soil highest concentrations are expected for CeO$_2$ and TiO$_2$. Whereas, the predicted concentrations for quantum dots was the lowest of all investigated MNM due to a very small production volume. However future exposures of these materials may be considerably higher if production and use increases. For example the increased electrification of transport could lead to an increase in the use of quantum dots and carbon nanotubes in batteries.

Environmental concentrations of four MNMs in the EU (Sun et al., 2016)

The study used dynamic probabilistic material flow modelling to predict the flows of four MNMs (nano-TiO$_2$, nano-ZnO, nano-Ag and CNT) to the environment and to quantify their concentrations. The study included the following technical compartments: landfills, sewage treatment plants (STP), waste incineration plants (WIPs), recycling and export. The environmental compartments were atmosphere, natural and urban soil, sewage sludge treated soil, surface waters, and sediment.

The study indicated that in all the technical and environmental compartments considered, nano-TiO$_2$ had far higher concentrations than the other three MNMs. This reflected the greater production volume of this MNM. Most MNMs entering surface water end up in sediment and this showed accumulated concentration ranging from 6.7 µg per kg for carbon nanotubes to about 40 000 µg per kg for nano- TiO$_2$. In most cases the concentrations in Waste Incineration Plants are at the mg/kg level.

The researchers do stress that the reported concentrations do not consider any further chemical reaction such as dissolution or transformation, which will likely be very important for materials such as Ag and ZnO.

The use of dynamic models is standard in assessing flows of materials in situations with rapid technological changes and therefore also seems appropriate to MNMs. An example of ‘dynamic probabilistic material flow’ modelling used to estimate concentrations of four MNMs in the EU (Sun et al., 2016) is shown in Box 7.

Gottschalk et al.’s 2013 comparison of modelled and measured concentrations has shown that, within a certain range, there is some understanding of the expected environmental concentrations of MNMs. Although validation is minor, these can provide useful and urgently needed data for the environmental risk assessment of MNMs. The authors also point out a number of ongoing issues with modelling, which are discussed in Section 4.5. These are important to consider when interpreting the results but also to provide an indication for how future studies can be improved.

4.5 Knowledge gaps

4.5.1 Nanomaterial production and use

A major source of modelling errors lies in the uncertainty of the level of MNM production, and emission rates from products, as well as the market penetration of MNM products (Nowack et al., 2015). Researchers still find it difficult to quantify the amounts of MNMs produced and contained, used and consumed in products. Industry could help considerably in making exposure modelling more precise by providing figures to the public and the scientific community. As long as the companies are reluctant to inform academia on current and future quantities of MNMs produced and used, improving the robustness of exposure modelling by reducing uncertainties will remain difficult (Gottschalk et al., 2015). However, requests for data from industry
must be framed so as to provide meaningful answers, for example it should be specified that researchers are looking for data on different nanoforms and what definition they are using to describe that nanoform.

In 2012 Piccinno and colleagues estimated the worldwide and Europe-wide production of ten different MNMs (Piccinno et al., 2012). This was based on a survey sent to companies producing and using these MNMs. The survey also addressed information on distribution of the produced MNMs to different product categories (see Box 4.2). The survey produced by Piccinno and colleagues is a snapshot in time as the use of nanomaterials is ever changing, but it is still informative.

As part of their 2014 study, Sun and colleagues compared the production estimates of five MNMs with those used a few years previously and showed that generally the production figures had increased considerably (Sun et al., 2014). In order to counteract the issues (i.e. varying figures over time) a form of dynamic modelling can be used which is discussed in Section 4.4.2.

4.5.2 Estimates of concentration purely based on mass.

Current models provide estimates of concentration based on mass. This may not be sufficient when information on the material size, shape, surface chemistry etc. is also relevant to exposure. These properties are known to be important so there is a need to supplement mass-based units with indications of the material size, surface area etc.

Box 8. Survey results on production quantities and uses of MNMs (Piccinno et al., 2012)

The results reveal that some MNMs are produced in Europe in small amounts (less than 10 tons per year for Ag, quantum dots and fullerenes). The MNM with the greatest production figure is TiO2 with up to 10 000 tons of worldwide production. Between 100 and 1000 tons of CeO2, Iron Oxides (FeOx), Aluminium Oxides (AlOx), ZnO, and CNT nanomaterials are produced per year. The data for SiO2 cover the whole range from less than 10 to more than 10 000 tons per year, which demonstrates problems regarding the definition of which forms of silica are considered to be an MNM and how the mass is assessed.

The survey also obtained rough estimates for distribution to different product categories for seven MNMs, based on responses from industry. This shows, for example, that major uses for nano-TiO2 and nano-ZnO are in cosmetics. Although a rough estimate, the information is important for exposure assessment because it means that during this use a very high proportion of these MNMs end up in water or wastewater. For nano-Ag there are many uses, but most of them involve some contact with water (e.g. paints, textiles, cosmetics). Nano-CeO2 on the other hand has an important industrial use for a chemical polishing process and is therefore much more likely to have just a few point sources compared to, for example, the wide uses of TiO2, ZnO, and nano-Ag. Two other MNMs with uses in product categories where they are tightly bound are carbon nanotubes and quantum dots and therefore release during use is much less likely. However, for these MNMs the fate during end-of-life treatment becomes more relevant.

4.5.3 Lack of information on transformation processes and transformations

There is currently a lack of data on the transformation processes that are influential in environmental concentrations of MNMs. These transformation processes can be chemical, physical or biological. Chemical processes change the chemical structure, composition and/or the type of MNM. Physical transformation processes are those that influence the physical appearance of the MNM. Finally, biological transformations are defined as transformations processes that are biologically mediated.

In particular agglomeration is an important factor affecting the behaviour of MNMs in the environment e.g. for nano TiO2
agglomeration is considered in some models but not in others. In some cases the changes that occur to the initial form of MNMs can be very pronounced such as in the case of nano-Ag. For example, often the modelled concentrations of nano-Ag do not consider the almost complete transformation of nano-Ag into Ag-sulfides during wastewater treatment. Therefore, the modelled results may overestimate the environmental concentrations of nanomaterials remaining in their initial forms if they do not consider these transformations.

As part of the NanoDEN project in Denmark, Hartman and colleagues have identified the key transformation processes as described in Box 9.

### Box 9. Key transformation processes as identified by (Hartmann et al., 2014)

#### Photochemical transformation

- **Photochemical degradation**: Chemical change induced by light, which includes excitation of photocatalytic MNMs (absorption of a photon causing generation of free radical species) and photolysis of the MNM or components of the MNM (e.g. decomposition of coating material).
- **Oxidation**: The nanoparticle surface atoms or molecules are oxidized by loss of electrons. **Reduction**: The nanoparticle surface atoms or molecules are reduced by uptake (gain) of electrons.
- **Dissolution**: Process whereby a solid MNM dissolves (release of individual ions or molecules) in water. It increases with decreasing particle diameter.
- **Precipitation**: The process of dissolved species forming a solid phase (such as metal ions released from an MNM precipitating into a solid material).
- **Speciation / complexation**: MNMs (or released ions / molecules) associating with other molecular or ionic dissolved chemical substances in the environmental matrix. This includes interactions with macromolecules (e.g. chemisorption to the MNM surface, forming a surface coating).

#### Physical transformation

- **Agglomeration**: Reversible coagulation of primary particles to form clusters.
- **Aggregation**: Irreversible fusing of primary particles to form larger particles of the same material.
- **Sedimentation**: Process whereby MNMs in suspension settle out of the water phase.
- **Adsorption**: The association of the MNM with other solid surfaces in water. This can be divided into: MNM as sorbent: When other substances adsorb onto the MNM surface. MNMs as sorbate: When MNMs adsorb onto other surfaces.
- **Desorption**: Detachment of the MNM from other surfaces into water.

#### Biological transformations

Biologically mediated processes: processes whereby an MNM undergoes a transformation due to the presence of living organisms. This may include processes such as biological oxidation and degradation, interactions with bio-macromolecules excreted by organisms (e.g. leading to surface coating of the MNM).

transformation processes influencing the environmental fate and behaviour of MNMs (see Box 9). A schematic overview of these transformations is shown in Figure 5. It should be noted that some of the definitions of what these transformations (e.g. aggregation) entail do differ between authors and papers.

By reviewing the current literature, Hartmann and colleagues assessed the importance of these different transformation processes in terms of how they influence the behaviour of MNMs in the environment. They focussed on nine MNMs (Hartmann et al., 2014) and described the relative importance of the transformation processes. The MNMs were all in their non-coated form. This showed that the following processes were of highest importance for all MNMs regardless of chemical composition:

i) agglomeration / aggregation;

ii) sedimentation;

iii) adsorption of natural organic matter (NOM);

iv) sorption to other surfaces / retention in soil.

Fig 5: Schematic overview of possible transformations of MNMs in the environment. From: Hartmann et al., 2014. Environmental fate and behaviour of nanomaterials

They also ascertained which processes were important for which MNMs. For example, the transformation of Ag, ZnO and CuO was evaluated as being highly influenced by dissolution. For Ag and nZVI (nanoscale zero valent iron) oxidation and reduction processes are also of high importance. TiO$_2$ and CNTs (and to a lesser extent Ag and CeO$_2$) may undergo some photo-chemically induced transformations, which are considered of medium importance in the modelling of their environmental fate.

They then identified current knowledge gaps and compared this to the relative importance of the transformation processes in order to make a judgement on which knowledge gaps needs to be addressed urgently to help the development and improvement of exposure assessment of MNMs. These are quite specific knowledge gaps related to individual transformation processes such as sedimentation and dissolution.

The issue of transformation processes was also picked up by Dale and colleagues in their paper on modelling environmental fate of MNMs in aquatic systems (Dale et al., 2015). They pointed out that the main focus of modelling studies was on three processes: heteroaggregation, dissolution, and sedimentation. They suggest that up until recently models including these three processes have been relatively simple but models are developing rapidly and other processes, such as disaggregation, resuspension, and reactions with ligands such as natural organic matter, sulfide, phosphate, and chloride are likely to also be considered in the near future. As such more research is needed to understand these processes better and also what happens to the products of the transformation reactions, which could themselves have environmental impacts.

To address some of the gaps around transformation, degradation, interaction and transport of chemicals, Praetorius et al. (2012) have designed a flexible fate modelling framework, which can adapt to the specific properties of different nanoparticles in a river system. The results gained from running the model improve understanding of a nanomaterial’s fate and behaviour in a given environmental setting. They also analyse the findings regarding heteroaggregation between TiO$_2$ and suspended particulate matter in the Rhine River, and conclude that both the properties of the particulate matter, and the attachment efficiency (the affinity of TiO$_2$ and particulate matter) affect the transport potential of the nanoparticles significantly. (Praetorius et al., 2012)

Dale et al. also suggest that environmental conditions such as pH, temperature, availability of oxygen or sulfide will affect rates of transformation. Again scientific insight is needed to quantify these impacts as currently they are rarely considered in models. In addition the role of surface coatings and natural organic matter on the fate of MNMs is not very well understood and requires more research. This was also mentioned by Hartmann and colleagues who did not consider coatings in their analysis of transformation processes (Hartmann et al., 2014).

4.5.4 MNMs only constitute a portion of the nanomaterials existing on earth.

Nanomaterials are not only manufactured but exist in the environment naturally or occur accidently as a side effect of another process. Many so-called ‘bulk-derived nanomaterials’ have already been detected in the environment. For instance, soot, as part of the black carbon continuum, is formed by incomplete combustion of fossil fuels and vegetation. Its particle size in the nanometer to micrometer range falls partially within the nanomaterial domain. The existence of these nanomaterials that have not been manufactured purposefully can make estimating and modelling exposure difficult as it is a challenge to get an accurate estimation of their level.
4.5.5 Calibration and validation of models

There is a lack of measured data to calibrate and validate the models. In their review of modelling studies in 2013, Hendren and colleagues state this is one of the biggest challenges in developing and implementing exposure assessment models for MNMs. As such they suggest that the most valuable methods are those that account for uncertainty and allow for flexibility in the types of information incorporated (Hendren et al., 2013).

There are continuing improvements being made in the state of modelling and, with these, its value and contribution to nanosafety will increase. Some believe this work should not be hindered too much by knowledge gaps as it is important to find a balance between the need to know more and the pragmatic view of having tools that can be used within a workable timeframe (Peijnenburg et al., 2015).

4.6 Current and future developments: nanomaterials exposure under project MARINA

The EU-funded project MARINA aims to develop a more integrated systematic approach to handling health and environmental safety assessment and management of MNMs. Rather than doing this on an individual basis, the project plans to assess and manage overall risks for types or classes of MNM based on their physico-chemical properties. One of its four themes is ‘Exposure’ which aims to develop tools and methods to assess the release of MNMs and exposure in terms of the environment and human health. The work of Project MARINA in this area is described in more detail below.

4.6.1 Release scenarios: occupational, consumer and environment

MARINA has developed a framework to test the release of MNMs and assess the likelihood of release over the whole product life cycle. A literature review has been conducted to evaluate the characteristics influencing the release of MNMs. In addition, test procedures are being developed to collect information on emission and emission rates for different types of release processes such as sanding, drilling, sawing and weathering.

One of the published outputs was the ‘Summary report on processes and activities related to NM lifecycle within small and large industries’ (Roberts & Fort, 2014). The report describes in detail the material characteristics that influence the release of airborne particles from handling powders, liquid suspensions and nanocomposites as well as the processes and energies required.

4.6.2 Environmental behaviour assessment

Project MARINA has developed a model that combines experimental data and model approaches regarding the properties of the MNMs and the environments that influence the behaviour of MNMs. It has investigated specific MNMs in surface water, sediment, soil and sewage sludge. From this it has developed a protocol to assess the potential for MNMs to accumulate in organisms (bioaccumulation) from their properties or characteristics.

One of the outputs from the MARINA project is a review of the properties and processes determining the fate of MNMs in the aquatic environment (Peijnenburg et al. 2015). This examines literature and modelling studies to provide a general introduction on the most important processes of MNMs that influence what happens to them in the environment. It is supplemented by case studies on specific classes of MNMs, reporting current information on emissions...
and production and current knowledge on the important processes in the aquatic environment. The review also provides a description of both the particle-specific properties and the water characteristics that need monitoring to allow for better modelling of exposure to MNMs in water. By considering classes of MNMs instead of individual MNMs it also takes a step towards grouping and classifying nanomaterials so that exposure can be estimated from the category to which they belong rather than an individual assessment.

4.6.3 Environmental exposure assessment

This part of the exposure theme of the MARINA project has developed environmental fate models for MNMs based on material flow modelling. This includes methods to analyse trace amounts of MNM in complex environmental matrices using a type of mass spectrometry that can detect particles at very low concentrations.
5. Hazard: toxicology and ecotoxicology

Once an organism is exposed to nanomaterials, the biological effect depends on the ability of nanomaterials to reach its organs and cells. As such, detection of the uptake is essential in evaluating the safety of nanomaterials and any potential harm they may cause. Like other substances, the uptake of nanomaterials depends on how they exist in the environment i.e. as free particles and their aggregates and agglomerates, or bound in another substance (such as reinforcements in plastics) or distributed in a liquid (such as constituents of lubricants or oils).

5.1 Toxicology

The study of harm caused by substances in humans is known as toxicology. In humans there are three basic pathways for nanomaterials to get into the body:

1. via the air during inhalation;
2. via the digestive tract or orally;
3. via the skin or dermal uptake.

Nanoparticles can become stuck in the lungs, cause allergies on the skin or affect our digestive system, particularly in terms of the microbiome or natural bacteria. If the particles reach the blood stream they then have the potential for uptake and accumulation by different cells and organs in the body.

5.2 Ecotoxicology

The study of harm to the environment is known as ecotoxicology. Ecotoxicology is a multidisciplinary field, which integrates toxicology and ecology to study the effects of toxic chemicals on biological organisms as well as at the larger scale of ecosystems.

In principle, the same conditions apply for the uptake of nanoparticles to humans and environmental organisms but because of the diversity of species and how they interact with their environment there are many more possible routes of uptake than those that occur in humans, for example through gills rather than lungs. There is also much more variation in the source of the nanomaterials as it depends on the preferred habitat of the organism whether that be in water, soil or air. For example, in the case of plants, MNMs could potentially be taken up by roots and transported to shoots through vascular systems and this would vary depending on the plant anatomy.

As discussed in the previous section, nanoparticles released into the environment can change in many different ways and interact with other components of the environment. In the water they may be transported or bind to natural organic matter (NOM). In soil and air, they can form larger assemblies (agglomerates) with natural occurring nanoparticles or bind to other organic compounds. Agglomerates may reach a size and weight outside the nano-size and sink down in air and water. All these processes determine if and how environmental organisms come into contact with nanoparticles and whether they are taken up.

5.3 Nanoecotoxicology

A sub-discipline of ecotoxicology called nanoecotoxicology has emerged that specifically aims to identify and predict effects of nanomaterials on ecosystems (Schirmer & Behra, 2013). In order to deal with the combined complexity of both ecotoxicology and nanomaterials, nanoecotoxicology must recruit expertise from several disciplines: physicists and material scientists to understand particle structure; environmental engineers to quantify particle release and how they get in contact with the environment; chemists to understand particle behaviour; and biologists to quantify bioavailability and toxicity for different types of organisms (Klaine et al., 2012).

In addition, nanoecotoxicology requires different or adapted testing methods to ecotoxicology. In the initial rush to understand the potential hazard of nanomaterials, they were treated in the same way as traditional chemicals. That is, scientists used the same bioassays (methods to determine the activity or effect of a substance) and the same endpoints (the point signifying the completion of a reaction that is marked by a chemical change) (Klaine et al., 2012). They also assumed that nanomaterials behave in the same way as soluble contaminants. However quantifying the effect of nanomaterials in the environment has proven to be more difficult than previously experienced with soluble chemicals. Many of the traditional methods are proving inadequate and do not adapt well to the unique properties of nanomaterials.
The arrival of MNMs has prompted new ways to study toxicology and ecotoxicology. Science needs to evolve to monitor the unique ways in which MNMs behave and to assess their impact. Unrine reports there is growing awareness that it is not necessarily the properties of the MNMs that govern their toxicity, but how these properties evolve as the materials change in ecosystems and within organisms (Unrine, 2014). In other words the environmental and biological history of the MNMs will influence their subsequent effects. Alongside awareness of the importance of environmental transformations, new methods have been developed to predict and quantify exposure and bioavailability (Unrine, 2014).

Overall the difficulties in conducting actual measurements has resulted in an increase in modelling studies. In addition the ever-expanding number of nanomaterials and products containing nanomaterials has led to a need for a new form of study which uses profiling methods to classify nanomaterials into groups according to their relevant properties so their level of hazard can be ascertained from the group they belong to. So-called grouping and read-across methods will be explored in more detail in Section 5.7.2.

5.4 Current understanding of nanoecotoxicology

Research data indicate that some insoluble nanoparticles can pass through the different protective barriers or organisms, disperse themselves in the body, and accumulate in several organs such as the lungs, brain, liver, spleen, and bones (Hegde et al. 2015). However the picture of ecotoxicity of MNMs is still far from clear. There is still a great deal that science can do to clarify the picture and develop new methods that are more appropriate to nanotechnology.

There has been a wide range of research on ecotoxicology and toxicology of MNMs. This includes studies on cells in culture (in vitro), microbes, invertebrates, vertebrates, plants and complex collections of species. Unsurprisingly more research is done in vitro and with smaller animals as these are easier to study and offer more concrete results. There is also a global drive to reduce research with animals. These different approaches offer different strengths but have also sometimes resulted in different conclusions regarding the bioavailability and toxicity of nanoparticles (Choi et al. 2014).

Clearly not all organisms can be tested for harmful effects from nanomaterials and the choice of which ‘model’ organism to use is important. Different organisms have different advantages and can help understand the different routes that nanomaterials take (see Box 5.2). There are of course organisms that have been used widely in standard toxicity testing but these may not be so relevant to nanomaterials because of their individual properties and behaviour (Selck et al., 2016). Summarised below is an assessment of the research on ecotoxicity in the aquatic and terrestrial environments. As is the case with industrial chemicals in general, there is very little done on airborne nanomaterials, apart from toxicity in industrial workplaces.

5.4.1 Aquatic environment

According to Canesi and colleagues, the number of publications on the ecotoxicity of MNMs in aquatic invertebrates has exponentially grown in the last few years (Canesi & Corsi, 2016). Their review focused on marine species’ reactions to a range of nanoparticles (particularly polystyrene, but also titanium dioxide, cadmium chloride, gold, silver, silicon dioxide, zinc oxide and cadmium sulphide quantum dots) and they reported that the percentage of studies in this area has also risen considerably (up to 38% of the total). The most studied group are bivalve molluscs, which make up about half of the studies, whereas less attention has been focused on sediment-dwelling invertebrates (benthic infauna) that make up only a fifth of studies. Their review of evidence indicated that the effects of several different types of nanoparticles on marine invertebrates may be particularly apparent in the immune system. For bivalves the main modes of action appear to be oxidative stress and cell injury in proteins, membrane and DNA damage. There is also evidence that several
of the nanoparticles studied (polystyrene NPs, silver NPs, SWCNTs, SiO$_2$, fullerenes and zero-valent nanoiron) adversely affect embryo development in marine invertebrates. The researchers indicate that, for metal-based NPs, the observed effects may be due to metal ion solubilization in sea water; however, other, particle-specific mechanisms may also be at play. They posit that particle coatings may contribute to toxicity, possibly owing to the better colloidal stability or differences in uptake routes in different types of embryos; overall, there is still little information on the causes of these developmental effects.

Schultz and colleagues review studies looking at nanotoxicity more generally in aquatic systems and it focuses on three priority nanomaterials: TiO$_2$, ZnO and Ag (Schultz et al., 2014). Their review indicated that there was a lack of research comparing toxicity of these three MNMs to their bulk counterparts. There were only such comparative studies for TiO$_2$ which showed that seven out of nine forms of (nano) TiO$_2$ are more toxic than their micrometre-sized counterpart.

Upon comparing the three MNMs with each other, the review suggested that TiO$_2$ nanomaterials are most toxic of the three MNMs to algae; toxicity was dependent on size of the MNM and principally associated with whether and how the MNMs bind to the organism. Whereas for Zn and Ag nanomaterials it is the process of dissolution and subsequent release of their ionic metals that appears to represent the primary mode of toxicity to aquatic organisms.

More recently, researchers are also studying how nanomaterials may affect food chains in the aquatic environment and an example is given in Box 10.
Box 10. Case study: Toxicity and transfer of coated silver nanowires in an aquatic food chain consisting of algae, water fleas, and zebrafish (Chae & An, 2016)

Nanomaterials of various shapes and dimensions are widely used but information on toxicity of silver nanowires (AgNWs) to freshwater organisms and their transfer through the food chain is limited. AgNWs are used for electrical conductive applications in products such as solar cells and displays. The study aimed to evaluate the toxicity of 10- and 20-µm-long AgNWs to the alga *Chlamydomonas reinhardtii*, the water flea *Daphnia magna*, and the zebrafish. It also studied the movement of the AgNWs through this three-species food chain.

Results indicated that AgNWs directly inhibited the growth of algae and destroyed the digestive organs of water fleas. The results showed that longer AgNWs (20 µm) were more toxic than shorter ones (10 µm) to both algae and water fleas, but shorter AgNWs accumulated more than longer ones in the body of the fish.

Overall, this study suggests that AgNWs are transferred through food chains, and that they affect organisms at higher trophic levels, potentially including humans.

5.4.2 Terrestrial environment

Compared to research with aquatic organisms, there is currently less research on the effects of MNMs on terrestrial organisms (Bouguerra *et al.*, 2016). Most of the research has been conducted on plants, investigating effects on their germination and growth. In terms of plants, studies have illustrated that MNMs can have both negative consequences (e.g. oxidative stresses induced by MNMs) and positive consequences (e.g. nano fertiliser in a relative low exposure range) on agricultural crops (*Ma et al.*, 2014).

For animals, the majority of research has been on invertebrates living in the soil, for example the *Caenorhabditis elegans* which is a type of roundworm (see Box 11). Researchers have also started investigating possible effects on food chains in the terrestrial environment (see Box 12).

5.5 Species sensitivity distributions

One of the criticisms of *in vitro* and *in vivo* studies is that they do not consider biological impacts in a truly realistic context. Although there are an increasing number of examples of studies investigating toxicity in isolated food chains this does still not account for the complexity of ecosystems in nature. The increasing amount of data from laboratory toxicity tests with MNMs can be used to generate species sensitivity distributions (SSDs). These model the range in sensitivities to chemicals of different species and provide an estimate of the potentially affected fraction (PAF) of species that will be harmed from exposure to MNMs. This can then be used to establish threshold concentrations, which, when exceeded, suggest that action should be taken. For example, the lower fifth percentile of the SSD indicates that 95% of species are not harmfully affected by an MNM and so most species should be safe (see Garner *et al.*, 2015).
Box 11. In vivo studies with *Caenorhabditis elegans* (Choi et al. 2014)

*Caenorhabditis elegans* is a type of roundworm about 1 mm in length and with an average life span of 2–3 weeks. There are several attributes that make it particularly useful for studying ecotoxicology including its short reproductive life cycle, large number of offspring and ease of maintenance.

In their review Choi and colleagues concluded that studies on *C. elegans* have yielded a wealth of insight into the relative harm of various nanoparticles (Choi et al. 2014). Research has provided understanding of their mechanisms of toxicity or how the MNMs actually cause harm and the importance of physiological barriers in modulating their effects. Compared to *in vitro* research in cell culture, the concentrations of nanoparticles required to cause toxicity are higher and more comparable to those observed in other studies of whole organisms. This indicates that physiological barriers such as skin, provide a protective mechanism that is not obvious in research in cell culture. Research on *C. elegans* has also improved knowledge on the interaction of MNMs with environmental variables such as natural organic matter and on aging, indicating that these can have dramatic effects on toxicity. For example, fulvic acid which is a form of natural organic matter significantly decreased the toxicity of silver nanoparticles to *C. elegans*, in some cases reducing mortality from 100 to 0 %.

Despite the many advantages that *C. elegans* may have over other organisms, Choi et al. do stress that there will always be a need for complementary investigations in other systems as no single model organism is sufficient. For example, *C. elegans* lacks lungs, and may therefore be a poor model for nanomaterials that have a very high height-to-width ratio, such as carbon nanotubes, that might exhibit asbestos-like toxicity. Earthworms may be more suitable for studies looking at the uptake and elimination of nanoparticles because of their larger size.

5.6 Knowledge Gaps

5.6.1 Better knowledge on properties and interactions

There is growing recognition that a useful approach to assessing the hazard of MNMs is to classify them into groups and predict environmental implications (see Section 5.7.2). As such there is a need to improve information on their intrinsic properties and gain a deeper understanding of their interactions.

For example to evaluate the biological effects of nanoparticles there needs to be greater understanding of their interactions with cells and organelles — and with molecules that are involved in the maintenance and metabolism organisms, such as polysaccharides, proteins and colloids — and of how these interactions affect their toxicity (Canesi & Corsi, 2016). Research has shown that nanoparticles can interact with plasma proteins in the biological fluids of mammals to form a protein corona at the surface of the nanomaterials. This may affect toxicity, but there is a lack of knowledge of the impact, or whether such processes could occur in the external environment (Canesi & Corsi, 2016; Selck et al., 2016). There is also a lack of knowledge on the effects of particle coatings on the properties and toxicity of nanomaterials and the processes by which they attach to media such as soil and sediment as well as biological surfaces (Unrine, 2014). The discovery of such effects shows that there is a possibility that large quantities of scientific data, gathered in ignorance of these effects, are now not useful. There exists the potential for novel hazards, both acute and long-term (Dawson in SFEP 2015) in such cases, specific regulatory restrictions beyond that of comparable materials, which don’t exhibit such interactions, may be required.
Box 12. Ecotoxicity of TiSiO$_4$ for terrestrial species (Bouguerra et al. 2016)

Bougherra and colleagues focussed on one nanomaterial in their study: titanium silicon oxide (TiSiO$_4$) as previous studies had indicated that this nanomaterial had important effects (Bouguerra et al. 2016). TiSiO$_4$ is used in optics, glass and ceramic applications. The study investigated the impacts on two species of invertebrate — the earthworm (*Eisenia Andrei*) and the springtail (*Folsomia candida*). It analysed the effect of TiSiO$_4$-contaminated soil on their avoidance behaviour and on their reproduction. It also investigated effects on seed germination and seedling growth for four plants: *Avena sativa* (oat), *Zea mays* (corn), *Lactuca sativa* (lettuce) and *Lycopersicon lycopersicum* (tomato).

The results showed that earthworms demonstrated avoidance of soil contaminated at the highest concentration of TiSiO$_4$ (1000mg per kg) but that there was no significant effect on the reproductive function of either invertebrate species. Although an increased in avoidance may not be thought of as harmful to earthworms, it is ecologically relevant. If earthworms avoid a soil then the ecosystem services provided by these organisms, such as soil aeration, will be compromised.

There were also significant effects on the growth of lettuce and tomato plants. These effects are important since they may affect ecosystems in two ways. Firstly through the direct impacts on the plant communities and the ecosystem services they provide and, secondly, there may be indirect effects on populations that eat these plants.

5.6.2 Methodological improvements

- Numerous researchers have pointed out the need for more ecotoxicity studies based on realistic environmental scenarios (Kühnel & Nickel, 2014; Canesi & Corsi, 2016; Krug, 2014). For example, focussing on marine environments Canesi & Corsi stress that the majority of studies have looked at species living in the water column and more information is needed on those species living in close contact with sediments, where deposition of MNMs, and consequent concentrations, may be higher (Canesi & Corsi, 2016; Selck et al., 2016).

- In addition, there should be a focus on reporting important chemical components such as pH, ionic strength and the presence of nutrients or proteins that may alter the physiochemical properties of MNMs. UV-mediated MNM transformations have the potential to enhance the toxicity of the MNMs to aquatic organisms; therefore, future studies should consider the effects of UV exposure on toxicity levels (Schultz et al., 2014).

- Toxicologists must consider effects not just of pristine MNMs, but also of environmentally modified MNMs and those that have aged (Kühnel & Nickel, 2014; Choi et al., 2014; Selck et al., 2016). Carbon nanotubes, for example, may be partly encapsulated by polymers if they were released from a polymer nanocomposite. Thus, the form that may reach the environment after usage or disposal of products may differ from that which is tested by scientists (Selck et al., 2016).

- Researchers should also attempt to characterise nanomaterials throughout the duration of the toxicity experiments (start, middle and end). This will provide researchers with a clear indication of any alterations in the properties that may have occurred during the investigation (Schultz et al., 2014).
There is a need to decide on the best model organisms to use to determine ecotoxicity. So far the use of different model organisms has produced varying conclusions regarding the most important factors influencing toxicity (Unrine, 2014).

Suitable controls are essential in any MNM toxicity study but will depend on the specific type of MNM being investigated. For example using a bulk material (micrometre sized) control should also be considered for metal and metal oxide nanomaterials (Schultz et al., 2014).

There is a need for standardised measures to facilitate the toxicity testing of MNMs and help risk assessments to be conducted more efficiently (Petersen et al., 2015). A proactive approach is required to develop these measures that can be used by industry and policy to ensure helpful safety evaluations (Hegde et al., 2015).

5.7 Current developments

5.7.1 Project MARINA: ecotoxicology

As part of project MARINA (Work Package 10), researchers evaluated the practicality and validity of existing ecotoxicity tests, such as the systems used by the OECD, to assess MNMs. They have investigated their effectiveness on different parts of the environment such as water, sediment and soil and on different test organisms and protocols.

Eight OECD test guidelines were adapted based on the testing of at least one ion-releasing nanomaterial (Ag) and two inert nanomaterials (TiO$_2$), and the researchers present their proposals for the modification of the following toxicity tests:

- growth inhibition using the green algae *Raphidocelis subcapitata* (formerly: *Pseudokirchneriella subcapitata*; TG 201);
- acute toxicity with the crustacean *Daphnia magna* (TG 202);
- development toxicity with the zebrafish *Danio rerio* (TG 210);
- impact on reproduction of the sediment-living worm *Lumbriculus variegatus* (TG 225);
- impact on activity of soil microflora (TGs 216, 217);
- and impact on reproduction of invertebrates *Enchytraeus crypticus* and *Eisenia fetida* (TGs 220, 222).

For every test system the protocol was developed individually as the demands of the various test organisms differ. Recommendations for different tests included the omission of materials that reacted differently with the NMs, pH and duration adjustments, use of exposure chambers coupled with water changes every 24 hours in order to improve NM dispersion, testing on life-stages of fish that were found to be more sensitive to NMs, more clearly defined criteria for sub-lethal effects and reduction of organic matter in soil to reflect real conditions. The researchers assume that the described approaches and modifications will be suitable for the testing of further NMs with other chemical compositions.

The research has provided a general overview of ecotoxicity effects and a ranking of the MNMs tested. The aim is to forward this information to the relevant standardisation organizations to improve test guidelines for the testing of MNMs.

5.7.2 Grouping nanomaterials

A key question remains: is it necessary to perform assessments of each individual material, or can a grouping approach be adopted? A ‘grouping’ approach has been established for many chemicals already, where testing on particular toxicological endpoints is performed on certain substances, and the results are considered applicable for other closely related compounds. Finding MNM properties that can be used to group MNMs would enable the assessment of an NM with unknown toxicity using data determined for other MNMs in the same group. Grouping would also require less reliance on testing, which would reduce the need for in vivo studies and help meet the international requirements to reduce animal testing (Oomen et al., 2015).
The nanoparticles, concentration and exposure time, were correlated with ROS release to investigate the possibility of grouping according to these properties. The results indicated that this approach is feasible for metal oxide nanoparticles but might need reconsideration and a larger data set for CNTs. More specifically, SiO$_2$ and ZnO show decreasing ROS release with increasing size. The other nanomaterials showed no clear correlation but, for these nanomaterials, the amount of data was insufficient or other factors than the ones investigated in this study influenced the ROS release such as agglomeration or aggregation. In terms of concentrations there was an increase in ROS release with increasing concentration for all metal oxide nanoparticles apart from CeO$_2$. This indicates that most metal oxides could potentially be grouped according to size for those endpoints where ROS is an appropriate indicator for a common mechanism leading to an adverse outcome.

Researchers in project MARINA (see Section 5.7.1) suggest that approaches that decrease the workload for conducting groups of tests under an overall strategy, or within an individual test should be explored further. They state this could include options for not conducting a test using decision trees with specific criteria, or a reduced number of treatments and/or concentrations within a protocol, or a short test duration. The researchers also see scientific value in approaches for grouping and ranking and for efficient ‘concern-driven’ testing strategies; along with in silico or in vitro approaches, these may help in avoiding animal testing altogether.

5.7.3 NANOSOLUTIONS project

The NANOSOLUTIONS project aims to determine the ‘biological identity’ of MNMs and develop a computer program that can use the properties of an MNM to predict their ability to cause health or environmental hazards.

The overarching aim of NANOSOLUTIONS is to provide a means to develop a safety classification for MNMs. It will identify and better understand the characteristics of MNMs that determine their
potential to cause biological hazard. This includes damage due to interactions at the molecular, cellular, and organism levels. By understanding the fundamental characteristics of MNMs that underpin their biological effects, the project aims to provide a sound foundation for classifying MNMs according to their safety. The resulting MNM SAFETY CLASSIFIER will allow for the transition from descriptive toxicology to predictive toxicology.

5.7.4 Systems toxicology

‘Systems’ toxicology is a term indicating a paradigm shift from judging toxicity based on indications of one observed biological effect, such as impact on development or reproduction, to judging toxicity based on a range of molecular and functional changes across the whole biological system under consideration. Biological systems can be populations of organisms or systems at the level of the actual organism e.g. the circulatory or respiratory system. It integrates in vitro and in vivo toxicity data with computational modelling, developed from methods found in systems biology.

The approach gathers quantitative information on specific molecular changes that occur after exposure to nanomaterials from in vitro and in vivo research. Mathematical models are then built to describe these processes across the biological system under consideration. The integrated data analysis leads to the identification of how biological networks are impacted by exposure and enables the development of predictive mathematical models of toxicological processes. This perspective integrates current knowledge, computational analysis, high-throughput experiments, and the potential for improved screening, prioritisation and risk assessment.

Project MARINA includes a work package on systems toxicology, which aims to bring together findings from other work packages to allow a parallel analysis of the different data sources. Another Nanosafety Cluster project, SUN: Sustainable Nanotechnologies (FP7), ran a course on analysis of large amounts of data gained by high-throughput techniques for screening nanomaterials in 2014.

5.7.5 Intelligent testing strategy (Farcal et al., 2015)

It is clear that a large number of variables determine the biological impact of nanomaterials. As discussed in ‘grouping’ some consider it to be unfeasible to evaluate each nanomaterial individually regarding hazardous and physico-chemical properties. This debate continues as others regard the best and safest approach is to test MNMs individually and by adopting techniques such as Intelligent testing strategies (ITS)

Farcal et al. (2015) suggest the development of an intelligent testing strategy (ITS) to allow risk evaluation of MNMs — which is an approach already encoded in schemes such as REACH. An ITS integrates data from in vivo tests, in vitro tests, computer models and physicochemical properties as efficiently as possible — which not only means reducing costs, but also minimising uncertainty and minimising animal testing. It is the use of all available data and intelligence to acquire missing knowledge. As such, in vitro tests are especially relevant in an early phase of an ITS for screening purposes and for steering decisions for the choice of subsequent steps.

As part of the MARINA project Farcal et al. conducted a study to identify and evaluate in vitro test methods for toxicity assessment to facilitate the development of an intelligent testing strategy (ITS) (Farcal et al. 2015). They analysed six MNMs and considered ten different assays to assess toxicity, alongside a thorough description of the different properties of the MNMs. Their study indicated that this range of data could be deployed for the development of an ITS suitable for risk assessment of MNMs, as well as providing a rich source of data for modelling.

While the concept of ITS has potential, in particular when considering parallel assessment of larger number of materials, consideration needs to be given to the practicalities of implementing this approach, as industry will tend to be interested in only acquiring data on the nanomaterial they are developing or have developed and not on other MNMs that might inform (or benefit from) the ITS.
6. Risk assessment

Risk assessment refers to the methods used to understand the potential consequences caused by exposure to harm (Walker et al., 2015). More specifically it quantitatively estimates the probability that a substance will cause harm in a particular situation or situations. As such risk is a product of exposure and effect (Sips et al., 2015). Figure 6 provides more detail on the elements of risk management.

In their review of approaches to assess the environmental impact of nanomaterials, Klaine and colleagues describe risk assessment as being ‘where the rubber meets the road in terms of translating science into societal action’ (Klaine et al., 2012). Risk assessment provides policymakers and managers in industry with a summary of the relevant scientific information about nanomaterials that they can then integrate with socioeconomic and political information to produce policies to manage the production and use of nanomaterials.

Koelmans and colleagues discuss approaches to risk assessment for MNMs in aquatic environments (Koelmans et al., 2015). They argue that only prognostic risk assessments (PRAs) are possible i.e. those that evaluate the future risk of MNMs not yet released into the environment. Retrospective risk assessments (RRAs) that look at observed effects are currently not possible because the concentrations of MNMs are too low and adequate methods of observation are not available.

Their review concludes that sufficient tools exist to perform risk assessments for MNMs at a screening level. Although there has been a lot of development in exposure models to incorporate the main features known to affect MNM behaviour, they have yet to be validated against field data. For toxicology data, cost-effective screening could be based on intelligent testing strategies that anticipate gaps in information and fill them by grouping processes that then assume similar data for MNMs in that group and more detailed evaluation could be done using species sensitivity distribution modelling which is discussed in Section 5.5.

Box 13 (next page) provides an example of a risk assessment conducted by Coll and colleagues using modelled exposure data and hazard data from species sensitivity distribution modelling (SSD).

As with assessing hazard and exposure, the main challenge for risk assessment is whether it is possible to apply current risk assessment paradigms to the case of nanomaterials (Klaine et al. 2012). The rapid development of MNMs means there is increasing variety in terms of size, shape, structure etc. This makes risk assessment a challenging process since full testing of the wide range of slightly different MNMs may lead to unreasonable costs and at the same time may challenge the intention to reduce animal testing (Bos et al. 2015).
6.1 Existing risk assessment methodologies (Rickerby et al., 2015; Rio-Echevarria & Rickerby, 2015)

Rickerby and colleagues have recently reviewed existing risk assessment approaches for nanomaterials, analysing both strengths and limitations. This was done in two publications and four examples that are reviewed in both are listed in Table 5 (next page).

In one review the tools are classified as control banding, exposure assessment or comprehensive risk assessment. Control banding is the least thorough. It allows identification and prioritisation of risk and can be helpful to identify cases where there is concern and to flag up when a more detailed risk assessment is needed. Examples are the Precautionary Matrix and the Nanocommission Assessment Tool. Exposure assessments focus exclusively on exposure of workers to nanomaterials during manufacture and therefore neglect exposure of end users to products containing nanomaterials and the general public.

In general these two types of approach require fewer and more easily accessible data than a full risk assessment. For example the Nano Risk Framework which allows a complete analysis of potentially high risk nanomaterials requires quite stringent data which, even when they exist, are often not accessible for the assessor outside the registrant-regulator domain as they are commercially sensitive. Although some results are available from modelling studies, they still require scientific verification. In comparison the Precautionary Matrix is more user-friendly and provides visual results in the form of radar charts.

6.2 The MARINA risk assessment strategy (Bos et al., 2015)

MARINA’s risk assessment strategy provides guidance for an efficient generation and collection of data on MNMs, whilst at the same time ensuring a reliable assessment of risks.

The strategy was created for different users, for example those working in industry during the design-phase or policymakers within a regulatory framework. As such flexibility is required to address the different assessment goals of the users.

The MARINA Risk Assessment Strategy is composed of two phases and four pillars which are closely linked (see Figure 7).
Table 6: Four examples of available nano-specific risk assessment tools

<table>
<thead>
<tr>
<th>Methodology/tool</th>
<th>Format</th>
<th>Approach type</th>
<th>Health</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precautionary Matrix</td>
<td>Web application</td>
<td>Control Band</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Nano Risk Framework</td>
<td>Questionnaire</td>
<td>Risk Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>RA of Manufactured NM</td>
<td>Reports</td>
<td>Risk Assessment</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>NanoCommission Assessment Toll</td>
<td>Questionnaire</td>
<td>Control Band</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Phase 1: Problem framing. The main goal of this phase is to identify Relevant Exposure Scenarios (RESs) throughout an MNM’s life cycle and to verify whether exposure may potentially lead to adverse effects. It sets the scope for Phase 2 by defining information and/or testing requirements to be addressed and by providing guidance for the strategy to collect this information in a flexible and efficient way. Of course it will be impossible to foresee all possible future exposure scenarios (e.g. the possible applications/uses) but it is recommended to address as many scenarios as is reasonably possible.

Phase 2: Iterative risk assessment. This process of consists of four steps:

Step (a) Risk characterisation. This step focuses on whether the question on potential health and/or environmental risks can be addressed. If yes, the risk can be characterised by using tools from the risk characterisation pillar. If no, go to step (b) which focuses on defining data needs.

Step (b) Defining data needs. This step identified the data gap(s) with the highest priority to be addressed, and defines the data or information that is required to fill in these gaps.

Fig 8: Schematic overview of the MARINA Risk Assessment Strategy (Bos et al., 2015)
Step (c) Data gathering. Compilation of data is based on formal guidance, guidelines and other tools to ensure reliability and relevance.

Step (d) Data evaluation. Evaluation of collected data and existing data, including consideration of possibilities for read-across, grouping and data-sharing.

Phase 2 is repeated until a risk characterisation can be performed and/or additional data collection is not expected to further refine the risk assessment. The level of detail of the final risk assessment and acceptable uncertainty will depend on the goals of the users and the framework in which the risk assessment is performed.

The strategy is a flexible approach to risk assessment and, as would be expected, it has advantages that help it achieve its goal but also some issues that still need to be resolved which are listed in Box 14. As it is used and applied these issues are likely to be addressed, as are other complications that may arise.

6.3 Life-cycle analysis

Life cycle analysis (LCA) provides a broad, holistic measure of environmental impact throughout a product or system’s life cycle.

Although LCA may be used to complement risk assessment, unlike risk assessment the objective of LCA is to quantify the overall environmental performance of the product or material. This means it does not just consider the negative or risk aspects to the environment but also the possible environmental advantages. A wide range of environmental impacts are taken into account, which can be local (e.g. ecotoxicity), regional (e.g. acidification) and global (e.g. climate change, resource use). A common unit is used, such as CO₂ emissions, which allows there to be a comparison between different products, particularly between the traditional product and the nano-alternative. This can help inform decision making on a policy and industry level.

6.3.1 LCA studies on MNMs

The number of LCAs for MNMs is not huge but it is growing. In 2014 Miseljic and Olsen reviewed 29 such studies (Miseljic & Olsen, 2014). They found

Box 14. Advantages and issues of MARINA Risk Assessment Strategy

Advantages

- The main advantage of this strategy is the iterative approach allows flexibility to generate data for a user’s purpose.

- The strategy is exposure-driven, i.e. focusing on defining relevant and realistic exposure scenarios.

- Another important aspect of the strategy is the integration of exposure, fate and hazard throughout the whole process, especially during data-gathering and for characterising risk.

- The final scope and level of detail of the risk assessment and the associated uncertainty may depend on the user’s goal. These goals can range from a full risk assessment within a regulatory context to a targeted testing in an MNM design-phase or risk assessment for a specific exposure scenario such as a specific application of an MNM.

Issues to be resolved:

- An important issue that remains to be solved is the identification of points of decision. Development of guidance for these decision moments is required, including decision criteria and the assessment of associated uncertainty.

- Another issue concerns how to assess uncertainty qualitatively and/or quantitatively. In addition, guidance is needed on how to define the most important data and the choice of appropriate tools or sets of tools.

- Regular performance of case studies will be needed to verify the feasibility and practicability of the strategy and to identify where adjustment is needed.
there were similar data problems to other approaches, such as lack of knowledge about the physico-chemical properties of MNMs that influence their environmental behaviour and effects. There are also other barriers that need to be overcome to strengthen the value of LCAs for MNMs such as better and more comprehensive life cycle inventory data, especially for manufacturing and use, and the lack of data for nanospecific fate, transport and toxicity effects.

There is wide variation in LCAs of MNMs on a number of counts. Firstly on how much of the ‘life cycle’ they assess: some look at the whole life cycle from cradle to grave whilst others analyse only cradle-to-gate (from resource extraction to the factory gate, or when it is transported to the consumer) and focus on impacts caused by the manufacturing process.

Overall, from the results of the LCAs studied by Miseljic and Olsen, some general patterns were reported. On the whole, MNM production tends to have higher use of energy and resources in the production stage than equivalent bulk materials but an improvement in how it performs its function and its efficiency, which means less environmental impact in the use stage. This means that cradle-to-gate LCA comparison of products with and without MNMs tend to indicate that MNM products are more energy demanding and, therefore, have a worse cradle-to-gate environmental profile, for example when comparing socks manufactured with and without nanomaterials (Meyer et al., 2010). However cradle-to-grave LCA comparison of products with and without MNMs indicate that the use phase is better for MNM products as an improved functionality is usually achieved, e.g. when comparing clay-propylene nanocomposites with steel or aluminium in light-duty vehicles (Osterwalder et al., 2006).

The review of the 29 studies suggested that much research only considers cradle-to-gate, which works only within the boundaries of the manufacturing process. There is a poor coverage of the use and disposal life-cycle stages.

### 6.3.2 LCA and risk assessment

LCA is one of the only tools currently available to understand the overall, holistic impacts of products and systems. One aim of a LCA, as posited by Peijnenburg and colleagues, is to inform and complement risk assessment, by estimating the potential exposure to MNMs throughout the life cycle of the selected products (Peijnenburg et al., 2015). In the US, the 2011 National Nanotechnology Initiative’s Environmental Health and Safety Research Strategy suggests LCA has an integral role to play and stresses the need for research to integrate life-cycle considerations into risk assessment and risk management processes. In response to this Walker and colleagues reviewed articles that applied LCA to nanotechnology (Walker et al., 2015). They stress that LCA gives a broader outlook than risk assessment and can more easily compare environmental burdens of different products or materials and identify environmental trade-offs. For example, LCA has shown that the use of nanomaterials could greatly reduce reliance on platinum group metals in automobile catalytic converters, metals that have been deemed critical elements.

Risk assessments can consider exposure scenarios at a specific time and place, whereas commonly applied LCA methods usually use average regional factors. Walker et al. suggested that risk assessment and LCA can work together, where LCA is used to identify hotspots followed up with a risk assessment to determine when and where such hotspots warrant interventions. They also point out that when LCAs are conducted there is a need for the results to be presented in a more audience-friendly package so they can be used practically for risk assessments and policy decisions.

### 6.4 Knowledge gaps

The validity of the predicted risks of MNMs to the environment hinges on two main aspects: the choice of the model for exposure assessment and the quality of the hazard data (Coll et al., 2015). As such, the knowledge gaps that have been identified in previous sections on exposure and hazard are also relevant here.

There is still a lack of standardisation with respect to test protocols and representative species groups to be tested. More standard tests are needed for selected groups of species that live in the range of environment compartments (Koelmans et al., 2015).

More risk assessment studies of complex ecosystems are required. These are needed to validate and calibrate more simplistic and practical predictions. Such tests are lengthy and costly, but provide highly relevant
information because they represent natural conditions as closely as possible. This also assures realism with respect to aging and changing of MNMs over time as well as the effects of MNMs on the complexity of food webs. (Koelmans et al., 2015)

In line with release and exposure assessment for risk estimations, in LCA there is a poor coverage of the use and disposal life-cycle stages in terms of the release of MNMs and the potential toxic impacts (Miseljic & Olsen, 2014). Current LCAs also tend to use generic life-cycle inventory data and assumptions rather than ones specific to nanomaterials (Miseljic & Olsen, 2014).

A general issue is that the lack of relevant and robust data leads to the assumption of worst-case scenarios and potentially the termination of projects developing nanomaterials. The NANoREG project is producing an exhaustive list of specific risks or risk areas for specific classes of products, industries and applications.

6.5 Current developments

6.5.1 Grouping and read-across approaches for risk assessment (Oomen et al., 2015)

Grouping and read-across approaches can be utilised to meet the double goal of efficient yet sufficient information gathering to assess the safety of human health and the environment. Oomen and colleagues identified three different possible applications of grouping and read-across for nanomaterials within the perspective of the MARINA Risk Assessment Strategy (RAS) (see Section 6.1).

- Firstly, nanomaterials can be grouped based on their variation in physicochemical properties in order to design a testing strategy that covers the entire group.
- Secondly, groups can be identified to guide the identification of further information needs and indicate possibilities for read-across early in the risk assessment process.
- Thirdly, read-across can be considered for data related to specific endpoints or toxicity, using information from a source material for the target nanomaterial.

These grouping and read-across approaches can improve the use of available information on nanomaterials and are flexible enough to allow future adaptations related to scientific developments.

In order to increase the robustness of grouping, practical application using case studies is needed to complement the process. Another important issue that still needs to be addressed is how to deal with the uncertainty of the grouping and read-across process. Furthermore, more details on decision-making and how the results of research based on grouping can inform decisions should be developed.

6.5.2 ProSafe project

There are many projects investigating the health and environment impacts of MNMs. The EU NanoSafety Cluster provides a forum for these projects, but a new overarching project called the ProSafe Project aims to evaluate and integrate the results of the most relevant EHS projects, translating them into building blocks for potential future regulatory actions. It will do this through two main products: a White Paper, and an agreement on long-term goals for EU-US collaboration.

The philosophy behind the White Paper is that policymakers and regulators throughout the EU and beyond, should be stimulated and supported to take up and implement the results of NANoREG and similar projects.

The White Paper will be a document that will provide building blocks for regulators and industry to address safety aspects of MNMs including evaluated methods for testing and assessing risks of nanomaterials and including Safe by Design (SbD).

The Joint Document will be the integrated assessment of the results of NANoREG and other EU initiatives (such as other nanosafety projects like MARINA, SUN etc.).

Supporting activities:

- Foresight Study: As input for the White Paper, ProSafe will analyse what will come in the next 3-10 years for nanomaterial product development and its risk management. This foresight exercise will seek knowledge of experts who are currently preparing products as well as those developing exposure and hazard assessment methods.

29 For more information see http://www.h2020-prosafe.eu/
30 For more information see http://www.nanosafetycluster.eu/
Establishing standard approaches for data management: To coordinate the development of tools allowing data sharing between databases to support model development of Safe by Design and for regulatory purposes.

Safe by Design (SbD): A novel safety culture will be developed that incorporates SbD as part of the core research, development and production activities of nanomaterial and nano-implemented products. See Section 6.4.3 for more detail on Safe by Design.

6.5.3 Safe by Design (SbD) - NANoREG project

According to the NANoREG project, SbD is an approach that incorporates the environmental health and safety aspects in an early stage of the innovation process of MNMs to guarantee safety for humans and the environment.

The concept is not new and has been in use by industry for some years. It aims to identify uncertainties and potential risks at the earliest possible time during an innovation project, as well as identify measures to reduce or eliminate these uncertainties and risks. It is not a stand-alone concept, but designed to be seamlessly integrated into current innovation processes.

The NANoREG project has identified SbD as part of its toolbox of risk assessment instruments of MNMs. The concept of SbD sits well with its objectives to develop testing strategies aligned with innovation process and to establish a collaboration between policy, industry and science.

Currently there is no standard definition of SbD and, as such, the understanding of the term is somewhat diffuse. NANoREG has produced a document that aims to define the concept more clearly with respect to MNMs (Sips et al., 2015). This aims to produce a common understanding of safety, uncertainty and risk so that the concept of SbD can be applied more easily. It does this in a realistic framework, stressing that absolute safety can never be achieved and risks can only be reduced and weighed against each other because avoiding one risk often leads to exposure to another risk.

Sips et al. point out that the SbD concept must be compatible with current industrial innovation processes in order to successful. As such the concept based around a model of the innovation process and they specify five ‘gates’ where risk assessment should be performed. This starts in the risk analysis part at the beginning and continues into the risk management process, meaning every activity carried out during a ‘normal’ risk analysis is also carried out within a SbD process (see Figure 8)

<table>
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<th>Gatekeeper question</th>
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<th>Sketch business case?</th>
<th>Go to development?</th>
<th>Go to test?</th>
<th>Launch?</th>
<th>Continue?</th>
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<td></td>
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<td>Stage 2</td>
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<tr>
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<td>Detailed investigation (build business case)</td>
<td>Experimental development</td>
<td>Testing and validation</td>
<td>Full production and market launch</td>
<td>Post-implementation review</td>
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</tbody>
</table>

Fig 9: Current industrial innovation processes and risk management (Sips et al., 2015)
7. Current and future challenges for the science-policy interface

7.1 Nanomaterials on the market

In a best-case situation, policy makers would have all the necessary information to regulate production and application of MNMs before they were placed on the market. However, research into risk assessment of MNMs has tended to lag behind the development of nanotechnology itself. Some MNMs have been in use for a long time, even before they were termed MNMs, e.g. carbon black and silica. Introducing new legislation that proposes new scientific risk assessment tools requires careful analysis of the best way to also apply it to already existing nanomaterials to ensure that any risk identified only via new methods would also be identified and addressed for existing materials.

There is, however, an outstanding question over whether and how the safety data generated previously (often with insufficient characterisation data according to current standards), can be applied. There may be updates required, depending on current understanding of the applicability of test methods employed, or changes in the interpretation of results, issuing from changes in the characterisation parameters of the materials over time.

Similarly, it is also important that the producers of new nanomaterials that share some properties with existing nanomaterials, can claim relevance of the data on existing MNMs in their risk assessments only after adequate consideration of all the facts applicable to their particular case.

7.2 Third-generation MNMs and beyond

We have only just begun to see the range and variety of materials that will enter markets in the future.

There are already 3rd-generation, or very novel MNMs in development. These are responsive and perform an active function. They can, to some extent, assemble or...
disassemble themselves. This means they can produce ‘smart’ materials that react to their environment and change accordingly. For example, in medicine 3rd generation MNMs have been developed to target the specific pharmacological site where a drug must be administered therefore reducing amounts of the drug needed and possible side effects.

According to Savolainen and colleagues, by the year 2020 4th generation MNMs will also be available (Savolainen et al., 2013). These are molecular nano-systems that allow the manufacture of molecular devices ‘by design’ where each molecule in the nanosystem has a specific structure and plays a different role (Klaine et al., 2012) Molecules will be used as devices, and from their engineered structures and architectures will emerge fundamentally new functions.

Within the plethora of what is to come, the potential for novel hazards cannot be excluded. This provides a challenge for risk assessment as it means there is likely to be an increase in complexity and cost to develop the methods for assessing an increasing number and variation of materials entering the market. Nanotechnology is a moving target and some suggest that methods for the assessment of safety of next generation nano-enabled products must shift to hit this target (Savolainen et al., 2013).

7.3 Nanomaterials as part of a product

As with research on industrial chemicals in general, most of the current safety research on MNMs aims to understand intrinsic properties and focuses on the behaviour of pristine MNMs rather than how they behave when they are in a matrix or part of a product. However with MNMs, the relevance of the environment and the material’s history is, due to their transformational properties, higher. In order to inform risk assessments effectively, research must also focus on studying nanomaterials in their more complex product environments.

As with all chemicals, once the nanomaterials are bound (for example, by a polymer matrix, to make a tennis racquet), the risk of exposure to the nanomaterial is reduced significantly — unless the matrix is subsequently subjected to abrasion. MNMs are usually modified by how they are used and what they are combined with. From a risk perspective, this means that what is released from a product may have little in common with the ingredients that went into it (The Graphene Council 2014). The situation is similar for many chemicals, which is why REACH requests that industry lists the uses of chemicals in the registration, requiring the specific chemical safety aspects of these identified uses to be assessed.

Potential differences in the level of risk for an MNM throughout its life cycle have been recognised for some time; back in 2004 the UK Royal Society’s publication Nanoscience and Nanotechnologies: Opportunities and Uncertainties explained that the dangers of nanomaterials before they are incorporated into a product might, for example, cause risk mainly to those working with the materials but not to the users of the final product (Dowling et al., 2004).

7.4 Closing knowledge gaps

Scientific understanding of nanomaterials and their safety is rapidly developing but there are still significant unknowns. For example, their impact on the environment over a prolonged period is yet to be extensively investigated. Identifying materials, or perhaps more importantly, the properties of materials, that have the potential for adverse long-term effects is a priority. However, as for chemicals in general, society will never reach a state of absolute scientific knowledge and it is likely gaps will continue to open when others have been closed.

One way that researchers are reducing the knowledge gaps is by increasing understanding of testing methods, for example, with regard to where nanomaterial-specific tests need to be developed and refined, and where conventional tests can be used. This will enable standardised methods, comparability and harmonisation of data. Laboratories implementing the standard tests for very persistent, very bioaccumulative (vPvB) persistent, bioaccumulative, toxic (PBT), or persistent, mobile and toxic (PMT) materials are also contributing to reducing these gaps.
The physicochemical properties of the various MNMs require further study: for example there is a need to supplement concentration estimates based on mass with information on the MNM size, shape, surface chemistry, where these properties are relevant to exposure. Transformations, such as agglomeration/aggregation, sedimentation, adsorption of organic matter, disaggregation and resuspension, as well as the effects of ageing and of environmental conditions (such as pH, temperature and availability of oxygen), also warrant further research and better understanding (a list of key transformation processes, and the transformations most relevant to particular MNMs, as identified by Hartmann et al. (2014) can be found in Section 4.5.3).

The understanding of nano-ecotoxicology has scope to be much improved. The properties of a bulk material may not be a good guide to the toxic or non-toxic effects of the nanomaterial, and some materials have the potential to have more severe or additional toxic effects in their nano form. This is especially the case, given new knowledge of nano effects that cross the protective barriers of organisms, and which opens up new possibilities for interactions of nanoparticles with, for example, cells, organelles and DNA, and with molecules involved in organisms’ maintenance and metabolism (e.g. proteins, polysaccharides and colloids).

There are a range of important, ongoing questions regarding MNM mechanisms of action. For example, there seems to be fewer studies that compare nanomaterials’ toxicity explicitly with the toxicity of their dissolved, micro, or bulk counterparts, than studies comparing MNMs with each other. Using bulk, microscale or dissolved materials as experimental controls might be employed towards this end in future — although not all materials can exist in the same chemical structure for both nano and larger types. To give another example question, regarding the production of more ‘environmentally friendly’ plant-, bacteria- and fungus-produced nanomaterials: how do the effects and properties of these differ from conventionally produced nanomaterials that may fulfil a similar function?

These and many other specific questions touch on a broader outstanding theme: how to group nanomaterials appropriately, so that hazard and risk can be estimated from the category to which they belong rather than only by an individual assessment. Developing appropriate grouping classifications is important; it will mean that testing, guidance, policy and legislation can be properly and usefully applied, and it would reduce the reliance on in vivo studies and on animal testing.

Overall, MNM test methods representing natural conditions are scarce. To assess MNM risks effectively, a better understanding of their effects and concentrations in complex and realistic situations is required. Such an increased understanding will enable a calibration with the large number of simpler and more specific
experimental results. Species sensitivity distribution studies — providing an estimate of the potentially affected fraction of species that will be harmed from exposure to MNMs — are just one way researchers might consider biological impacts in more realistic ecosystem contexts.

Improving exposure data (e.g. via greater understanding of interactions and mechanisms of action in the real environment) will also enable the development of more accurate and suitable exposure models. Some researchers identify a need for exposure models that account for uncertainty and allow for flexibility in the type of information that can be incorporated. High-throughput testing and screening, utilising systems biology approaches may increase the capability for risk-based prioritisation of nanomaterials. Additionally, life-cycle assessments of nanomaterials, incorporating use and disposal phases as well as the manufacturing phase would be useful, as would strategies that enable LCAs and risk assessments to work together in a mutually beneficial way.

In itself closing such knowledge gaps progresses our understanding and provides a basis for even further advancements in capability. The lack of robust and/or applicable data may lead to assumptions based on unrealistic scenarios, and potentially the termination of projects developing (and testing) nanomaterials, leaving the state of knowledge no further advanced. As more data is produced, and more comparisons made, however, we will gain a better picture of the mechanisms of action, and the models for predicting exposure will be improved accordingly. Indeed, despite the current knowledge gaps, more realistic models can already be developed, so that they will be ready for use once input data is available.

7.5 Meeting the challenges

While the breadth of issues may appear to be daunting there is every reason to suppose that a more thorough and deep understanding of nanomaterials and nanosafety is possible. The complexity of research into safety also requires an interdisciplinary approach that follows current, new and emerging research needs, technology and production closely (Selck et al., 2016).

Increasingly, commercial success will depend on innovating responsibly, which involves taking account of the environmental and societal benefits and impacts of a product as well as its technological and economical viability. One of the most enduring challenges to developing and using these new materials is to provide reasonable ways to assess the safety of nanomaterials, which are at least on a par with conventional chemicals. To facilitate responsible uptake of the most promising technology, researchers can further support progress by providing scientific support for the adequacy of assessment tools so that industrial developers can in turn provide evidence of demonstration of safety.

Reasonable boundaries need to be placed on what is considered safe enough, by establishing necessary research questions, and by ensuring that the necessary data to answer them are generated.


Peijnenburg, W.J.G.M. *et al.* (2015) A Review of the Properties and Processes Determining the Fate of Engineered Nanomaterials in the Aquatic...


Roberts, K. & Fort, K. (2014) Summary report on processes and activities related to NM lifecycle within small and large industries. Grant: 263215. MARINA_D_5.1.doc


Further reading

News Alert articles

Nanomaterial alternatives assessment: a powerful tool for identifying safer options (June 2017)
Judging whether to replace a hazardous conventional chemical in a product with a nanomaterial — i.e. to assess which is the safer alternative — is challenging for many reasons. A new study suggests that chemical alternative assessment frameworks could be adapted to better assess engineered nanomaterials with the help of new tools which provide data on hazards of, and exposure to, nanomaterials. [http://ec.europa.eu/environment/integration/research/newsalert/pdf/nanomaterial_alternatives_assessment_powerful_tool_identifying_safer_options_491na4_en.pdf](http://ec.europa.eu/environment/integration/research/newsalert/pdf/nanomaterial_alternatives_assessment_powerful_tool_identifying_safer_options_491na4_en.pdf)

Nanomaterial risk assessment frameworks and tools evaluated (March 2017)
A recent study has evaluated frameworks and tools used in Europe to assess the potential health and environmental risks of manufactured nanomaterials. The study identifies a trend towards tools that provide protocols for conducting experiments, which enable more flexible and efficient hazard testing. Among its conclusions, however, it notes that no existing frameworks meet all the study’s evaluation criteria and calls for a new, more comprehensive framework. [http://ec.europa.eu/environment/integration/research/newsalert/pdf/nanomaterial_risk_assessment_frameworks_tools_evaluated_484na1_en.pdf](http://ec.europa.eu/environment/integration/research/newsalert/pdf/nanomaterial_risk_assessment_frameworks_tools_evaluated_484na1_en.pdf)

Collecting data to explore the ecological threat of nanomaterials (October 2015)
The overall ecological impact of 10 engineered nanomaterials has been modelled for the first time using toxicity data from multiple living species. These models will allow researchers to assess the effect nanomaterials may have on both ecosystems and people. [http://ec.europa.eu/environment/integration/research/newsalert/pdf/collecting_the_data_to_explore_ecological_threat_of_nanomaterials_429na2_en.pdf](http://ec.europa.eu/environment/integration/research/newsalert/pdf/collecting_the_data_to_explore_ecological_threat_of_nanomaterials_429na2_en.pdf)

Future Briefs

Synthetic biology and biodiversity (September 2016)
Synthetic biology is an emerging field and industry, with a growing number of applications in the pharmaceutical, chemical, agricultural and energy sectors. While it may propose solutions to some of the greatest challenges facing the environment, such as climate change and scarcity of clean water, the introduction of novel, synthetic organisms may also pose a high risk for natural ecosystems. This future brief outlines the benefits, risks and techniques of these new technologies, and examines some of the ethical and safety issues. [http://ec.europa.eu/environment/integration/research/newsalert/pdf/synthetic_biology_biodiversity_FB15_en.pdf](http://ec.europa.eu/environment/integration/research/newsalert/pdf/synthetic_biology_biodiversity_FB15_en.pdf)

Identifying Emerging Risks (April 2016)
How can we better anticipate environmental changes? In our rapidly changing world, risks occur from ongoing changes (such as those occurring in the climate), to more sudden-onset risks, such as mutating microbial pathogens. This Future Brief explores some of the tools and approaches that can be used to identify emerging risk, including strategic foresight tools, citizen science and state-of-the-art monitoring technologies. [http://ec.europa.eu/environment/integration/research/newsalert/pdf/emerging_environmental_risks_early_warnings_FB12_en.pdf](http://ec.europa.eu/environment/integration/research/newsalert/pdf/emerging_environmental_risks_early_warnings_FB12_en.pdf)

Thematic Issues

Nanomaterials functionality (February 2015)

Integrating Environmental Risk Assessment (December 2015)
Environmental risk assessment is challenging because of the complexity of the physical and ecological systems around us. Natural disasters, the spread of dangerous substances, ecosystem changes leading to food and health security issues, and the emergence of new materials, new events and new knowledge make it essential to update our understanding continually, to be able to identify threats and opportunities for timely action. This Thematic Issue presents some collaborative and integrated paths towards forward-thinking assessment and management of environmental risks. [http://ec.europa.eu/environment/integration/research/newsalert/pdf/ship_recycling_reducing_human_and_environmental_impacts_55si_en.pdf](http://ec.europa.eu/environment/integration/research/newsalert/pdf/ship_recycling_reducing_human_and_environmental_impacts_55si_en.pdf)
ASSESSING THE ENVIRONMENTAL SAFETY OF MANUFACTURED NANOMATERIALS

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