Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations

Kai Savolainen (coordinator), Ulrika Backman, Derk Brouwer, Bengt Fadeel, Teresa Fernandes, Thomas Kuhlbusch, Robert Landsiedel, Iseult Lynch, and Lea Pylkkänen together with the members of the NanoSafety Cluster who have contributed to the document and listed in an alphabetical order in the Annex.
Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations

Kai Savolainen (coordinator), Ulrika Backman, Derk Brouwer, Bengt Fadeel, Teresa Fernandes, Thomas Kuhlbusch, Robert Landsiedel, Iseult Lynch, and Lea Pylkkänen
together with the members of the NanoSafety Cluster who have contributed to the document and listed in an alphabetical order in the Annex.
CONTENTS

Preface .................................................................................................................................5
Executive summary .............................................................................................................6
Strategic Research Agenda compact ................................................................................8
1. Introduction and background .......................................................................................34
   Consequences for the vision for 2015-2025 .............................................................38
   EU and international strategy documents ..................................................................39
   Elements of the strategy ............................................................................................42
   Towards understanding of nanomaterials .................................................................44
   Importance of nanosafety ...........................................................................................47
   Conclusions ................................................................................................................53
2. The nanosafety research landscape ..........................................................................58
   Nanosafety: facing the challenges .............................................................................58
   Action plans and strategic funding instruments .......................................................62
   European NanoSafety Cluster ..................................................................................65
   European stakeholder collaboration platforms .........................................................67
   International cooperation, standardization and education .........................................69
   Conclusions ................................................................................................................71
3. Prerequisites for nanosafety research ........................................................................76
   Regulatory framework .................................................................................................77
   Infrastructure ...............................................................................................................84
   Innovation and value chain .........................................................................................90
   Communication and dissemination ...........................................................................97
   International Collaboration .......................................................................................107
4. Common nanosafety research themes .......................................................................122
   4.1. Nanomaterial identification and classification .................................................125
   4.2. Exposure, transformation and the life cycle .......................................................146
   4.3. Hazard mechanisms, biokinetics, and vulnerable populations .........................162
   4.4. Risk prediction and management tools ............................................................180
5. Research priorities and roadmap ..............................................................................195
6. Implementation of the roadmap ..................................................................................204
Annex: Editorial group and list of contributors ..............................................................211
Preface

Nanotechnology will be one of the key technological drivers in building an innovation European Union (EU) based on smart, sustainable and inclusive growth. Nanotechnology has also been identified as the key-enabling technology (KET) for the EU. Nanotechnology has rapidly promoted the development of a new generation of smart and innovative products and processes that are nano-enabled, and have created a tremendous growth potential for a large number of industry sectors. It is important that this development continues so that all the useful properties of engineered nanomaterials (ENM) can be fully utilized in a number of nanotechnology applications.

The marked benefits brought about by ENM and nanotechnology applications have also created some concerns of their possible effects on human health and safety and environmental burden. A few observations on some potentially harmful effects of ENM have in some cases overshadowed the dramatic benefits of these materials and their nanotechnology applications. However, the real concern, rather than observations on some hazards of exposure to ENM, is the lack of systematic studies on hazards of or exposure to ENM. Hence, the true importance of this document is the identification of the knowledge gaps related to ENM safety, and directing the future research on ENM to enable the alleviation of the uncertainty, the real source of potential concerns associated with ENM and nanotechnology.

This document on the strategic priorities of nanosafety research during 2015-2025 has been produced as a joint effort of the European NanoSafety Cluster, a forum incorporating FP6 and FP7 funded nanosafety research projects. It also includes several nanosafety research projects, that have been funded by different EU Member States. It identifies four major areas of research that would greatly benefit our current understanding of ENM features, exposure to them, hazard mechanisms of ENM, as well as their risk assessment and management. Hence, the strategic vision on the future directions of European nanosafety research presented in this document may have a major impact on the future nanosafety research within and outside the European Union, and consequently, on the success of nanotechnologies.

Kai Savolainen, Coordinator, NanoSafety Cluster Director,
Nanosafety Research Center,
Finnish Institute of Occupational Health, Helsinki, Finland
Helsinki, June 4, 2013
Executive summary

Nanotechnology has been identified as one of the key enabling technologies (KET) in Horizon 2020 thus underlining the significance of this field for Europe’s competitiveness and its ability to provide the innovative goods and services essential for meeting global challenges. In particular, nanotechnology offers substantial possibilities for improving the competitive position of the EU and for responding to key societal challenges. Ensuring the safe and sustainable development and application of the nanotechnologies is thus a key objective.

The aim of this document entitled “Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnologies” (Strategic Research Agenda; SRA) is to introduce a strategic vision for future research on the safe use and safe applications of engineered nanomaterials (ENM). The time horizon for this document is 2015-2025. The SRA has been developed by members of the European NanoSafety Cluster, a forum for ongoing FP6 and FP7 projects covering all aspects of nanosafety. The implementation of the SRA is expected to provide a major step forward in the development of safe and sustainable nanomaterials.

The goals of this document are to describe the current level of knowledge of the safety of nanomaterials and nanotechnologies, to identify knowledge gaps, and to set out concrete goals for the research on safety of ENM within the foreseeable future. In addition, an overview of the nanosafety landscape is provided. Nanosafety is seen as an integral part of the development of any novel nanotechnology or product; a multi-disciplinary and multi-stakeholder approach is needed to promote a culture of nanosafety in Europe and beyond.

Key challenges today are that available tools for the assessment of the safety of ENM are often inappropriate, or so laborious that adequate safety assessment remains highly problematic. Current resources or test methods are not likely to enable safety assessment of the numerous novel nanomaterials that are emerging at an ever increasing pace. This means that new safety assessment paradigms need to be developed during coming years to solve this problem. At the same time it is important to support regulators and the nanotechnology industry so that prosperity is maintained and current products are made safe for
citizens in Europe and elsewhere. This situation calls for rapid identification of research priorities and of a roadmap for nanosafety; we cannot afford to wait.

Several cross-cutting issues that need to be addressed in order to promote growth of the nanotechnology industry are identified in this document. These cross-cutting issues include: 1) the regulatory framework for ENM and nanotechnologies, coupled to the important issue of standardization to promote good practice and to facilitate communication; 2) the innovation/value chain for environmental health and safety and innovation and means to ‘unblock’ the value chain; 3) the development of infrastructures for nanosafety to promote research, education, and innovation; and 4) international collaboration and global dialogue, with a view towards a global research area in nanosafety, along with 5) communication and dissemination of research to key stakeholders beyond the research community, including industry, regulatory bodies, and others.

In addition, the SRA describes the current status and the research needs and priorities for the coming 10 years in four main thematic areas: 1) nanomaterial identification and classification; 2) nanomaterial exposure and transformation; 3) hazard mechanisms related to effects on human health and the environment; and 4) tools for the predictive risk assessment and management including databases and ontologies. The SRA concludes with a set of research priorities that are required in order to reach the goals of the roadmap. Ultimately, the successful and timely implementation of this roadmap – which is subject to further refinements as new research priorities emerge - will lead to the development of a nanoEHS (Environment, Health and Safety) tool box for exposure assessment, for hazard prediction, and for risk assessment and prediction as well as management that will allow the sustainable implementation of nanotechnologies. As an “enabling technology”, nanotechnology is applied early on and is a key element in the innovation/value chain. There is tremendous potential for nanotechnology to provide answers to societal solutions and it is therefore of critical importance to incorporate nanosafety into the development of novel nanotechnologies and products – safety before design.
Engineered nanomaterials promise remarkable benefits but their successful use requires resolution of potential health concerns

The aim of this document is to provide a strategic vision for future research to promote the safe use and applications of engineered nanomaterials (ENM). This goal takes on ever-increasing importance in respect of the rapid expansion in the production of ENM and products incorporating these materials. This development will lead ultimately to mass production of a number of engineered nanomaterials, and this will inevitably increase the exposure not only of workers but also of consumers to these novel materials. This development has also triggered increasing societal and public debate about the safety of ENM and associated technologies. These emphasize the importance of setting priorities and goals on research of safety of ENM, thereby minimizing the uncertainties around the safety and health issues surrounding these materials and nanotechnologies. Hence, the goal of this document is also to describe the current level of knowledge about the safety of ENM and technologies, to identify knowledge gaps, and set out goals for the research on safety of ENM. Identifying research priorities is essential if one wishes to achieve a set of concrete goals accompanied by time-lines and milestones with which to follow of the progress of the research efforts. In addition, the background and the current research landscape need to be surveyed. The time horizon set in this document is 2015 - 2025, from the start of the first research project funded by the “EU Horizon 2020” Framework funding programme for research and innovation, until the termination of the last project funded from that same programme.
Engineered nanomaterials and quality of life in Europe

The European Commission has recently (2011) adopted a recommendation on the definition of nanomaterial according to which ‘nanomaterial’ means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm. 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%. This definition is part of the regulatory environment in which also this document has to be operational.

The potential of ENM and nanotechnologies to improve the quality of life and to contribute to economic growth and competitiveness of industry is now widely recognized. Nanotechnologies can enable remarkable technological advances and innovations in many industrial sectors. However, there is an ongoing debate about the potential risks of ENM and nanotechnologies. In this context, it is important to consider that research and innovation have been identified as the key drivers of European social and economic prosperity. Nanosafety research is in a key-position to solve any challenges related to the concerns of ENM health or environmental effects and causing challenges to the promotion of these technologies.

Engineered nanomaterials, nanotechnology industry and safety

Competitiveness of the European industry lies at the heart of achieving these goals, and hence the role of innovations and the accelerated pace of the commercialization of innovations have been recognized as being fundamental in this respect. The recent Communication from the Commission on Horizon 2020 - The Programme for Research and Innovation emphasizes the importance of research and innovation for society at large. These considerations infer that there will be major changes in the future European research landscape and funding opportunities and all interested parties will need to adapt and prepare to meet these challenges. The new Programme for Research and Innovation, Horizon 2020 places a major emphasis on securing a strong position on key enabling technologies (KET) including nanotechnology and hence on engineered
nanomaterials. In particular, nanotechnology offers substantial possibilities for improving the competitive position of the EU and for responding to key societal challenges. Ensuring the safe development and application of nanotechnologies has been included in the broad line of activities of the Horizon 2020 proposal. The new technology applications not only should be safe themselves but should also offer substantial improvements to human health and environment protection while still remaining competitive. Due to the rapidly increasing production and use of ENM and utilization of nanotechnologies, it is self-evident that safety aspects must be fully understood and addressed.

**Key-issues of a strategy document aiming at achieving an impact**

The key elements of the strategy include the following:

1. Description of the current state of knowledge, the existing research landscape and identification of the requirements of the research environments and infrastructures essential for the promotion of research on safe ENM and nanotechnologies.
2. Identification of societal needs for the regulation of safety of these materials and technologies.
3. Identification of the necessary research goals for fulfilling of the societal needs and setting a time-line with milestones for the follow-up of the research progress.
4. Identification of the research priorities that will allow reaching the goals within the time-limit set by the strategy.
5. Identification of the means by which the results of the research can be disseminated, implemented and exploited to evoke a change in ways that will industry to promote the safe use of ENM and to guarantee the safety of workers and consumers, enabling regulators to make educated regulatory decisions.
6. Identification of the needs for further possible regulatory actions and possible further investments into infrastructures, educational and funding programs to be able to fully capitalize on the technological and economic benefits of these materials and technologies.
Towards a new toxicology for the 21st Century

Nanotechnology is hailed as one of the enabling technologies to innovation. Nanosafety, in turn, is concerned with the safe and sustainable development of nanotechnology. Without nanosafety research, widespread use of nanotechnologies in many sectors of society may well be slowed down and could even come to a complete standstill.

It is important to understand that we are still dealing with first generation of nano-enabled products (i.e. passive nanostructures) but it is likely that we will soon be confronted by the second generation products containing active nanostructures, and then to third generation systems of integrated nano-systems and, finally, by the year 2020 according to some predictions, to fourth generation products or heterogenous molecular nano-systems that allow the manufacture of molecular devices ‘by design’. This means that methods for assessment of the safety of next generation nano-enabled products also must evolve: nanotechnology is a moving target and researchers in the nanosafety field cannot afford to be aiming at a target that no longer exists.

Some challenges we are facing in the science of nanosafety related to exposure and hazard research have been resolved but others still remain. Those e.g. for nanotoxicology are not unique to this sub-speciality of toxicology but there is an urgent need for a “new” toxicology; toxicology for the 21st century. A proposal for a new, systems biology / toxicology approach was put forward in a 2007 report by the US National Academy of Sciences on behalf of the US Environmental Protection Agency (EPA). The overall aim was to promote a shift from toxicity testing primarily in animal models to \textit{in vitro} assays and \textit{in vivo} assays using lower model organisms, along with computational modeling, thus enabling the evolution of toxicology from being an observational science into a predictive science. The central part of this novel toxicology is to describe toxicity pathways which lead to understanding the molecular fundamentals. It has also been argued that “the testing of substances for adverse effects on humans and the environment needs a radical overhaul if we are to meet the challenges of ensuring health and safety.” In fact, it has been provocatively stated that “there is almost no other scientific field in which the core experimental protocols have remained nearly unchanged for more than 40 years” and that this will require that an entirely new system be urgently developed, even to the extent that it may need to be built from scratch, incorporating and benefitting from modern
methods and state-of-the-art technologies. It may be worthwhile noting that an evolving scientific discipline such as nanotoxicology is optimally positioned to take on board these new approaches.

We still lack a fundamental understanding of how nanomaterials interact with living systems and, thus, we are not yet in a position to assess the relevant end-points for nanomaterial toxicity. At the same time, we are faced with a tsunami of new materials for which testing or screening of toxicity is required. To resolve this situation, innovative methods for prediction of nanomaterial toxicity are needed.

**NanoSafety Cluster – coordination of nanosafety research in Europe**

The European NanoSafety Cluster is a DG RTD NMP CSA initiative to maximize the synergies between the existing FP6 and FP7 projects addressing all aspects of nanosafety including materials, hazard, databases, modeling and dissemination (see figure below). Synergy among the various FP6 and FP7 projects on nanosafety and other national projects, collaboration for maximizing impact, policy elaboration, planning of future actions, and international cooperation are the main aims of the European NanoSafety Cluster. About fifty projects dealing with nanosafety have either been completed or are running under FP6 and FP7. These projects, together with a significant number of nationally supported projects, represent valuable efforts of the scientific and industrial research community towards improving our understanding of these complex interactions. Information on all current or recently completed projects is collected in the NanoSafety Cluster Compendium.
Organization of the European Nanosafety Cluster

The main objectives of the European NanoSafety Cluster are to:

- facilitate the formation of a consensus on nanotoxicology in Europe
- provide a single voice for discussions with external bodies
- avoid duplicating work and to improve efficiency
- improve the coherence of nanotoxicology studies and harmonize methods
- provide a forum for discussion, problem solving and planning R&D activities in Europe
- provide industrial stakeholders and the general public knowledge on the risks of nanomaterials for human health and the environment

Synergy among the projects, collaboration for maximizing impact, and international cooperation are the main aims of the European NanoSafety Cluster.
Nanosafety research requires good governance

The International Risk Governance Council (IRGC) is an independent organization based in Switzerland that is focused on developing the concept and practice of risk governance. IRGC recently (2010) published a report on “The Emergence of Risks: Contributing Factors” in which it is postulated that emerging risks arise from a “fertile ground” that is cultivated by twelve contributing factors of which “scientific unknowns” is one factor (see figure below). It is noted that “communication” has a particularly key role, as it can influence all the other factors.

Twelve common factors contributing to emerging risks.

The twelve factors should not be interpreted as discrete units but as complex, interdependent factors. Moreover, the document proposes that the attribution of cause(s) to the emergence of risks should be examined via both reductionist and holistic approaches. The latter “systems perspective” approach focuses on describing the system as a whole and not as the sum of its parts.

Three independent scientific committees provide the European Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The committees also draw the European Commission’s attention to the new or emerging prob-
lems which may pose an actual or potential threat. The three committees are:
the Scientific Committee on Consumer Products (SCCP), the Scientific Com-
mittee on Health and Environmental Risks (SCHER) and the Scientific Com-
mittee on Emerging and Newly-Identified Health Risks (SCENIHR) and all are
made up of external experts. In addition, the European Commission relies on
the work of the European Food Safety Authority (EFSA), the European Medic-
cines Evaluation Agency (EMEA), the European Centre for Disease prevention
and Control (ECDC) and the European Chemicals Agency (ECHA). SCENI-
HR published a report in 2007 on the appropriateness of the current risk as-
sessment methodology for new and existing substances for assessing the risks of
nanomaterials. The aim was to assess the “fitness for use” of the risk assessment
methodologies described in the chemicals legislation for the risk assessment of
nanomaterials, and to provide proposals for improvements. EFSA published a
guidance document for the risk assessment of engineered nanomaterial applica-
tions in food and feed in 2011. That report is the first to give practical guidance
for addressing potential risks arising from applications of nanotechnologies in
the food and feed chain. In addition, the European Chemicals Agency (ECHA)
published extensive guidance on regulatory risk assessment of nanomaterials
under the European Community Regulation on chemicals and their safe use
(REACH) in April 2012. This guidance documents are additional to the general
guidance of Information Requirement and Chemical Safety Assessment under
REACH.

Nanotechnology is a multi-disciplinary field involving the skills of scientists
in disciplines such as material science, physics, chemistry, biology, engineering,
toxicology, clinical medicine, and social science. Similarly, nanosafety research
also depends on close cooperation between material science, biology, and toxic-
ology and risk assessment. This should be taken into account in the education
of the next generation of nanosafety experts.

Positive environment for nanosafety research is crucial

If one wishes that the industries producing ENM and products incorporating
these materials, as well as industry sectors utilizing nanotechnologies are going
to flourish, then we need to provide a favorable environment to allow these com-

mercial entities to be able to take the necessary risks to bring these new innovations into successful marketable products. Hence, if one wants to promote the success of these key-enabling technologies (KET) as identified by the European Union communication on the new Programme for Research and Innovation - Horizon 2020, favorable environments for this research have to be created to lay a foundation for such goals. One needs to address which are the major success factors that enable flourishing nanotechnology industry. This document has identified several cross-cutting issues that are absolutely necessary for ensuring success.

**Impact of chemicals and occupational safety regulations**

Chemical safety regulation in the EU is a structure based on two pillars. The first pillar is the legal framework for placing chemicals on the market, and the second is created from specific provisions for health, consumer, occupational safety and environmental protection. The regulatory framework can strongly support safe use of ENM provided that such goal will become a clear regulatory target.

i) According to the information given in the Communication “Regulatory Aspects of Nanomaterials” all nanoparticles in chemical substances must meet the requirements of the REACH (Registration, Evaluation and Authorization of Chemicals) (Regulation (EC).

ii) General requirements in relation to occupational safety and health of workers at workplaces are presented in the Council Directive 89/391/EC. The aim of this framework directive is to ensure a high level of protection of workers at work. The Council Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work describes the minimum requirements for the protection of workers from risks to their safety and health arising, or likely to arise, from the effects of chemical agents, including ENM, that are present at the workplace.
Infrastructure for nanosafety research is highly important

Promoting those infrastructures that support nanosafety research within the European Union is a prerequisite for the competitiveness of European nanotechnology research, innovations and industries. This will require institutional support for organizations with permanent financial funding from their own governments, i.e. to research institutes, universities or industrial research laboratories.

According to the European Commission Capacities Programme, the term ‘research infrastructures’ (RI) refers to facilities, resources and related services used by the scientific community to conduct state-of-the-art research in their respective fields. Examples include singular large-scale research installations, collections, special habitats, libraries, databases, biological archives, clean rooms, integrated arrays of small research installations, high speed communication networks, data infrastructure, networks of computing facilities, as well as infrastructural competence centres which provide a service for the wider research community based on an assembly of techniques and know-how. In short, the term research infrastructure means building, required research facilities and equipment, management structures of such infrastructures, and competences which are required for successful implementation of research – in this case of nanosafety research. The EU should provide opportunities to support these infrastructures and that may require adapting the current rules for funding.

Options for EU nanosafety infrastructure – a proposal for the future

Considering the options that would most effectively enable setting up of infrastructures to conduct European Union-wide nanosafety research, one possibility would be a single-site highly equipped facility with capacities to serve other EU nanosafety research facilities in strategic research areas. These types of investments could be situated in a stable organization with guaranteed fundamental resources into the foreseeable future, meaning that a long-range planning would be important.

Another possibility would involve networking of high-quality nanosafety research organizations, i.e. organizations with suitable space and laboratories, research equipment, human resources and competences, national stable funding and existing administrative support in research organizations within Euro-
This latter option, which would be potentially realistic at the European Union level, could be executed in collaboration with the European Commission and the European Union Member States, and their existing nanosafety research organizations. All the parties should have a vested interest in supporting this activity. This endeavour could lead to the identification of a network of competence centres, i.e. research centres capable of meeting a series of relevant quality requirements. The establishment a European Union wide Virtual Competence Centre Network on nanosafety would allow a better integration of European competences targeting nanosafety research. This type of Virtual Centre based on a network of separate organizations would also mean that one could envisage the establishment of an EU NanoSafety Research Centre which would have a relatively light administrative structure e.g. One important benefit of such an approach would be that the associated organizations would benefit from long-term basic governmental funding from their own EU Member State that could be supplemented by EU research funding to promote the efficacy of this kind of joint undertaking.

**Innovation, value chain, and nanosafety research**

The EU Flagship Initiative “Innovation Union” aims to improve framework conditions and access to finance for research and innovation in order to ensure that innovative ideas can be turned into products and services that create growth and jobs. The Flagship “An industrial policy for the globalisation era” aims to improve the business environment, notably for SMEs, and to support the development of a strong and sustainable industrial base able to compete globally. At the heart of these activities is the concept that Europe will undergo an industrial transformation based on scientific and technological leadership and excellence.

Deployment of nanotechnology is a major driver for the trend to improve existing products by creating smaller components and better (in both functional and environmentally-friendly terms) performance materials. Engineered nanomaterials (ENM) and the technologies which utilise ENM represent one critical pathway to achieve these goals.
Environmental Health and Safety (EHS): a barrier to innovation?

The current debate, including the lack of regulatory clarity and, in particular, the uncertainty surrounding the potential risks of ENM have had a negative effect on the development, uptake and exploitation of ENM in the European domain and have been identified as a major barrier to innovation based on these technologies. This has limited the extent to which these materials have been exploited through the value chain. The result of this phenomenon has been a failure to fully exploit the potential benefits associated with ENM throughout innovation chain in Europe. One shall, therefore, to overcome these barriers since this will make it possible to open up these value chains and realise their full economic potential. This goal can only be achieved through the development of a sound science-based foundation from which one can build a trustworthy and affordable safety framework.

The EHS programme of NMP research is not currently well integrated into the innovation-led FP7 work. Although many of the NMP projects have industrial partners, these projects are more fundamental in their nature and are concerned with achieving an underlying knowledge, models and tools for subsequent application in risk assessment and management. This activity is not only critical as a way to underpin the knowledge base, but it is also important that EHS research should be organized to make it more directly linked to the development of new materials, processes or products. Appropriate solutions will help to alleviate public concerns that that neither their health nor their environment will be harmed; this will be best achieved by clearly identifying those materials and applications for which there are absolutely no safety issues. This will dramatically open up the possibility of widening the range of ENM and applications, free from concerns about potential safety issues.

Communication and dissemination widely are required to assure impact of nanosafety research

Targeted, neutral and reliable communication by the different stakeholders associated with nanosafety can markedly enhance the acceptability of safe and trustworthy ENM and associated technologies and to promote a new safety culture in nanotechnologies. Key-stakeholders include regulators, industry, various interests groups, representatives of media and the public at large. Public confidence in nanotechnology is crucially important if these products are to achieve
commercial success. Successful dialogue, dissemination of reliable information on nanosafety, and outreach to various stakeholder groups will all help in assuring the general public and decision-makers that health and environment aspects are being taken into account. This will dramatically open up the possibilities of widening the range of ENM and applications while still maintaining consumer confidence. Hence, these activities will support safe and confident exploitation of ENMs in a wide range of products and processes for the benefit of Europe and its citizens, and being able to have a global impact. One of the key stakeholders could also be the Virtual European Nanosafety Research Centre, whose Coordinator could act as a hub of wide-reaching and global efforts to distribute neutral and balanced and trustworthy information on ENM and nanotechnologies within the European setting and globally.

**International Collaboration – nanosafety research is a global issue**

International collaboration may provide a fruitful platform for having a larger impact and obtaining benefits in research as well as in aspects related to governance and safety issues of nanotechnology. In fact, large projects involving a set of demanding multidisciplinary, hypothesis driven research endeavours require international collaboration because in most cases the required expertise or resources may not be available in any one single country. Furthermore, international collaboration has its merits also because ground-breaking innovations often take place in the interface or cross-roads of different scientific disciplines and research environments.

The globalization of research is proceeding rapidly and this is having significant implications for the European nanosafety research landscape. The forums of the production of new scientific knowledge are shifting from national to international arenas and comparisons of certain indicators across countries point to a positive relationship between measures of research collaboration and overall scientific impact.

International partnerships create unique opportunities for enhancing scientific excellence, physical and intellectual research environments and innovative training of young scientists. It The European Commission’s Nanosciences and nanotechnologies Action plan for Europe 2005-2009 has called for attention to and action on issues of mutual benefit at a global level such as nomenclature, metrology, common approaches to risk assessment and the establishment
of a dedicated database to share toxicological and ecotoxicological as well as epidemiological data. Progress has been achieved in many respects to identify the areas requiring joint efforts and the ways forward.

However, EU or global level coordination is far from achieving the goals of adopting international standards, nomenclature and databases, though important steps have been taken in that direction. Many obstacles or disincentives still exist, hampering collaboration across national borders and hindering the senior researchers or young talented investigators from working together. There is still a critical need to share knowledge in the health, safety and environmental aspects of nanotechnology.

Common nanosafety research needs

This document identifies four major research areas briefly introduced below, and they include: 1) material identification and classification; 2) exposure and transformation; 3) hazard mechanisms including both human toxicology and ecotoxicology; and 4) risk prediction tools including databases and ontologies.

Nanomaterial identification and classification

Most of the definitions of a nanomaterial concentrate solely on the size aspect (1-100nm), which misses the fact that nanomaterials are a very diverse group of materials with greatly varying properties. In order to enable prediction of impacts, a classification based on key parameters or biological interactions should be adopted.

The following approaches to group nanomaterials have been identified:

1. Classification by dimensionality / shape / morphology:
   Shape-based classification is related to defining nanomaterials, and has been synopsized in the ISO terminology.

2. Classification by composition / chemistry:
   This approach groups nanomaterials based on their chemical properties.

3. Classification by complexity / functionality:
   The nanomaterials that are in routine use in products currently are likely to be displaced by nanomaterials designed to have multiple functionalities, so called 2nd-4th generation nanomaterials.
4. Classification by biointerface:
   A proposal related to the hypothesis that nanomaterials acquire a biological identity upon contact with biofluids and living entities. Systems biology approaches will help to identify the key impacts and nanoparticle interaction networks.

**Approach for classification of engineered nanomaterials**

Sets of physico-chemical parameters that should be reported for nanomaterials have been identified. However, not all properties are relevant for all nanomaterials, many are not easily measured on a routine basis, and many are context-specific. Therefore, it is suggested that a distinction should be made between the *synthetic and biological identity* of nanomaterials. The *synthetic identity* describes the chemical, structural and compositional nature of the nanoparticles, and the *biological identity* describes the biomolecules that are absorbed onto the nanoparticles under specific conditions and the impact of these on the dispersion properties.

**The required research priorities on material characteristics include:**

1. Develop systematic sets of ENMs with properties varied in a stepwise manner that will allow assessment of the significance of each property for the toxicity of that ENM.
2. Describe “reference” states and agreed media compositions to enable identification of significant biomarkers and enable a move towards a predictive toxicity assessment.
3. Develop analytical methods that enable studying the longer term fate of particles following their interaction with living systems, i.e. complex matrices.
4. Developing risk assessment procedures that include the changes of ENM during their life cycle in a targeted manner.

It is essential that there are nanomaterial identification and classification approaches to determine the key descriptors that can be used to reveal correlations associated with impacts. The inter-relationship between the nanomateri-
als’ identification and classification is a cross-cutting topic in this whole document which feeds into the other nanosafety research themes.

**Exposure, Transformation and the Life Cycle**

Exposure of humans and the environment is a result of many sequential or concurrent processes. These facts have emerged from research related to ENM production, ENM characterization, aging of products containing ENM, human and environmental induced release of ENM into the environment, transport, transformation, degradation and possibly accumulation of ENM in the environment or along the food chain. The fundamental questions related to the existing frameworks have relevance to the now rapidly developing nanotechnologies, in particular those associated with the use of ENM, are:

- Is this existing framework appropriate to ensure the safe production, handling and use of ENM?
- Are the existing regulations and test guidelines applicable for testing and detecting the presence of nanomaterials, do they have to be adapted and/or do additions need to be made?

The current view is that the general existing regulatory frameworks are applicable but have to be adapted and extended for some ENM specific issues. It has been emphasized that ENMs are the subject of some special properties, especially those related to the transformation of materials during their life-cycle (LCA) or after their release into the environmental compartments which are known to alter their relevant substance characteristics e.g. size, shape, charge, state of agglomeration etc.

**General processes and areas of possible release and exposure:**

1. **Production**
   Possible release during production may occur through leaks into water and air in closed systems or open production processes.

2. **Handling and use**
   Handling and use covers several process-related stages e.g. handling of powders, diffuse emission from production plants, mechanical treatment of nanomaterials
3. Aging
Aging encompasses all processes taking place in the environment such as selective degradation, wash-out, increased brittleness of the material.

4. End of Life (EoL)
End of Life activities refer to activities related to
i) re-use or recycling;
ii) waste treatment, and
iii) disposal. In particular, during high energy processes, the release of nano-objects may not be excluded.

The required research priorities on exposure, transportation and life cycle include:

- Mechanistic understanding of processes determining the release of ENM.
- Understanding the transformation and transport of ENM.
- Understanding workplace, consumer and environmental exposure.

Hazard mechanisms, biokinetics, and vulnerable people

Hazard assessment of ENMs has made good progress during recent years, but knowledge is still lacking in many areas including modes of action and mechanisms leading to toxicity, identification of susceptible populations and vulnerable conditions, and aspects of biokinetics and its impact on toxicity. Mechanistic knowledge should be included in technology development, to help in the safe design of new ENMs in a bottom-up approach, and will feed directly into the development of a rational testing approach.
The key factors in developing knowledge and understanding the toxicity of ENMs are:

1. identification of the main modes of actions of toxicity for ENMs
2. understanding the transformation of ENMs during their life cycle and how this may influence their hazard potential
3. identification of the key physicochemical determinants that modulate ENM interactions and toxicity in biological systems

The required research priorities on hazard assessment include:

Hazard assessment enabling grouping of ENMs
1) Scientifically established grouping criteria
2) Understanding the association between material characteristics and the subsequent cellular events
3) Utilizing systems biology approaches in the prediction of ENM safety

Biokinetics including translocation and clearance
1) mechanistic knowledge resulting in groups of ENMs with marked similarities
2) bioaccumulative properties of ENMs and biokinetics

Susceptible populations and vulnerable conditions
1) Systematic research of ENM effects on susceptible populations
2) Systematic research of the effects of ENM on individuals with vulnerable conditions

Environment
1) Fate of ENMs in complex media and life cycle
2) Improved prediction of the (bio)degradation rate of organic nanomaterials
3) Development of standardized test methods for water environments and soil
Risk prediction and management tools

As the scientific basis of risk assessment (RA) for ENMs suffers from substantial limitations, both communication and dialogue are urgently needed with respect to risk management (RM) driven desired or approved actions. Databases and epidemiological or health studies can be considered as enabling ‘tools’, supporting the processes of RA and RM. Traditional risk assessment frameworks follow the four-step paradigm: 1) hazard identification; 2) hazard assessment; 3) exposure assessment; and 4) risk assessment.

The required research priorities on risk assessment include:

1. Development of ‘grouping’ strategies and nano-QSARs to predict relevant endpoints of toxicity and ecotoxicity
2. In vitro and in vivo (animals and man) risk assessment.
4. Characterization of the hazard in terms of quantitative dose-response relationships, relevant for threshold limit values.
5. Characterization of the hazard in terms of quantitative time-response relationships, relevant for the development of a reaction.
6. Globally harmonized epidemiological studies to validate biomarkers and to prevent/assess health effects in a longer perspective, and relevant field study approaches to assess potential effects of ENM at the population level of different environmental organisms.

To support the guidance in areas like risk management and decision-making, additional research will be needed in the fields of risk perception among many different targeted stakeholder groups and the main factors causing concerns.

The main achievement will be the development of integrated risk assessment and decision frameworks to enable forecasting the potential impacts of nanomaterials on human health and the environment and adequate risk management; this undertaking may require the development of novel risk assessment strategies that will replace the current version, being equally reliable, affordable but faster.
The research priorities on the risk management include:

1. Environmental impact on the basis of *in vivo*/*in vitro* toxicological studies and of physical/chemical properties of nanomaterials released into the environment.
3. Integration of LCA into risk assessment.
4. Integration of risk assessment into decision framework of risk management.
5. Integration of safe-by-design, closed production-to-product and green nanotechnology approaches into the development stages of new nanomaterials and their applications.

Research Priorities and Roadmap

The roadmap for nanosafety research 2015-2025 aims to provide an understanding of where the European nanosafety should be at the end of Horizon 2020. The roadmap also identifies the steps and achievements needed to achieve this aim within this time frame. This time horizon has been chosen based on the timing of the “Horizon 2020” Framework Programme for Innovation and Research; its first calls will open in 2014.

The milestones in the roadmap indicate the expected achievements of nanosafety research at different time points, at 5-year intervals, during 2015-2025. These milestones are presented for the four thematic priority areas separately in four tables below, and have been described in detail in the previous chapters. In each table, the research priorities have been grouped under larger heading, topics, that cover several defined research priorities.

The table on next page presents the roadmap, i.e. milestones 2015-2025, topics and research priorities in different topics at different time points under the thematic chapter on material characterization and grouping.
Nanomaterial identification and classification

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material classification</td>
<td><em>Definition</em></td>
<td>Classification systems in place</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Naming structure</em></td>
<td>Ontologies in place</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Characterization of ENM in complex matrices</em></td>
<td>Robust methods for ENM size determination</td>
<td>Methods for ENM surface characterisation</td>
<td>Methods for multicomposite ENM characterization</td>
</tr>
<tr>
<td></td>
<td><em>Test&amp; reference ENMs</em></td>
<td>Systematic sets of test ENMs ENMs certified in reference biofluids</td>
<td>Full datasets on test ENMs</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Validation</em></td>
<td>Validated labelled versions of test ENMs</td>
<td>Validation of key metrics for impact</td>
<td>Correlation of uptake, form and impacts</td>
</tr>
<tr>
<td>Measurement principles</td>
<td><em>Versatile methods</em></td>
<td>Versatile reference methods available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-nano-interactions</td>
<td><em>Biomolecules for uptake, transport etc.</em></td>
<td>Reference bio-interactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>NM impacts on protein function</em></td>
<td>Reference interactomes</td>
<td>NM properties leading to signalling disfunction</td>
<td></td>
</tr>
<tr>
<td>ENM engineering</td>
<td><em>Safety by design concepts</em></td>
<td>Safe design of new ENM in a bottom-up approach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENM metrics for hazard assessment</td>
<td><em>Key descriptors</em></td>
<td>Non-spherical descriptors defined</td>
<td>Full datasets on non-spherical reference ENMs</td>
<td></td>
</tr>
</tbody>
</table>
The table below depicts the roadmap related to the thematic chapter on exposure and transformation of engineered nanomaterials, and presents the different topics areas covering a range of specific priorities which compass the more detailed list of the priorities in this thematic area during 2015-2025.

Exposure and transformation

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Release and exposure</td>
<td>Mechanistic understanding</td>
<td>Process knowledge to allow the set-up of realistic laboratory simulation</td>
<td>Database on emission (per time) and release (per material unit) factors</td>
<td></td>
</tr>
<tr>
<td>Process dependent transformation</td>
<td>Transformation, mobility/transport</td>
<td>Gain knowledge on environmental mobility and transformation for computer simulation</td>
<td>Understanding effects of ageing on nano-objects</td>
<td></td>
</tr>
<tr>
<td>Exposure scenarios</td>
<td>Workplace, consumer and environmental exposure</td>
<td>Comprehensive, harmonized exposure inventories Exposure registries developed</td>
<td>Exposure models available Evaluation of exposure scenario models Models available for use of product cycle and exposure assessment</td>
<td></td>
</tr>
</tbody>
</table>
Within the topics, the subheadings then provide the separate research priorities in the four thematic areas separately. All the topic areas and research priorities have been drawn from the topic areas and research priorities presented in the four chapters presented above 1) material characterization; 2) Exposure assessment and release during the life cycle; 3) Hazards, biokinetics, and vulnerable populations; and 4) Risk prediction and management tools.

The milestones for 2015, 2020, and 2025, related, topics, and related research priorities under different topic separately are presented in a set of four tables.

The table below introduces the roadmap of the thematic chapter on Hazard mechanisms, biokinetics and toxicity testing, i.e. the roadmap for 2015-2025, the topics revealing several associated research priorities under this topic, and then the actual research priorities for the different time-frame separately.

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biokinetics and translocation</td>
<td>Prerequisites for research on ENM kinetics</td>
<td>Nanomaterial-specific analytical equipment available</td>
<td>Biokinetics integrated into toxicological testing</td>
<td></td>
</tr>
<tr>
<td>Hazard assessment</td>
<td>New approaches for ENM hazard assessment</td>
<td>Developing systems biology approaches using omics technologies</td>
<td>Development of appropriate QSAR models</td>
<td>A computational tool that can assess in the predicting of ENM safety</td>
</tr>
<tr>
<td>Vulnerable conditions</td>
<td>ENM and susceptible populations</td>
<td>Systematic research of ENM with known disorders</td>
<td>Validated in vitro models of appropriate ENM</td>
<td>Validated in vivo and ex vivo models for different diseases</td>
</tr>
<tr>
<td>Science-based regulatory approaches</td>
<td>Choice of test methods</td>
<td>Improved strategies for testing</td>
<td>Intelligent testing strategies available</td>
<td>Regulation</td>
</tr>
</tbody>
</table>
The table below introduces the thematic chapter on Risk prediction and management tools, and the relevant milestones to be achieved during 2015-2025, the wider topic areas under this thematic area, and the specific research priorities at different time points during 2015-2025.

The risk prediction and management tools

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk assessment</td>
<td>Pro-active risk management</td>
<td>Risk banding tools/ effective control measures development</td>
<td>High throughput screening approaches validated</td>
<td></td>
</tr>
<tr>
<td>Tools</td>
<td>Quantification of exposure reduction effectiveness</td>
<td>Testing and development of risk prioritization tools</td>
<td>RA-enabled LCA/ integration in decision tools</td>
<td></td>
</tr>
<tr>
<td>Epidemiology &amp; health surveillance</td>
<td>Health effect</td>
<td>Markers for short term effect identified</td>
<td>Markers for long term effect identified</td>
<td>Implementation of the markers</td>
</tr>
<tr>
<td></td>
<td>Register</td>
<td>Health surveillance registries developed</td>
<td>Using registries for research</td>
<td>Implementation of results for regulations</td>
</tr>
<tr>
<td></td>
<td>Study design</td>
<td>Pilot panel studies completed</td>
<td>Case-control studies completed</td>
<td>Longitudinal studies started</td>
</tr>
<tr>
<td>Databases</td>
<td>Infrastructure</td>
<td>Federated databases available</td>
<td>Format &amp; data quality standards set</td>
<td>IT procedures for automatic uploading</td>
</tr>
<tr>
<td></td>
<td>Ontologies</td>
<td>Ontologies in place</td>
<td></td>
<td>Automation of ontologies</td>
</tr>
</tbody>
</table>
The risk prediction and management tools (continued)

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk management</td>
<td>Risk perception and guidance</td>
<td>Development of risk communication strategies</td>
<td>Guidance on stakeholder concern assessment</td>
<td>Guidance on risk evaluation</td>
</tr>
<tr>
<td></td>
<td>Prevention through design approach</td>
<td></td>
<td></td>
<td>Integration of safe-by-design approaches</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>into the development stages of new nanomaterials and their applications</td>
</tr>
</tbody>
</table>

Implementation of the roadmap

Within the last 10-15 years, a number of novel basic methods to explore ENM-induced environmental health and safety (EHS) effects have been developed and validated. The implementation strategy proposed here is based on this existing knowledge. To facilitate and to enhance the advancement of nanotechnology, it is of utmost importance that we develop a successful implementation of a comprehensive and implementable scientific research agenda.

The most important key for a successful implementation is the excellence of the research proposed; this is aimed at meeting well identified and relevant priorities of the Strategic Research Agenda (SRA). The SRA shall be realistic in terms of goals and contents. Additional key elements of a successful implementation of such SRA for nanosafety research have been summarised in the key topics identified in the report of the National Research Council (2012).

Key steps for implementing the strategy:

- Infrastructure for implementation and accountability
- Evaluation of research progress and revision of the strategy
It is important to understand that all of the elements listed above are important prerequisites for the implementation of the research priorities and roadmap introduced in this document. In additions to those, also cross-cutting issues including a favourable regulatory framework, appropriate European Union wide infrastructure – preferentially a network of established competence centres working together in concert in the whole European arena should be established. Other crucial issues include a strong commitment from regulators to promote standardization, and the involvement of industrial partners that are willing to adopt the safety by design approach in their business thinking. Encouraging an awareness of the benefits of nanosafety is important because this makes it possible to clearly highlight the benefits of these new technologies and to communicate them in a tailored fashion to very varied and wide audiences i.e. ranging from the industrial representatives and regulators to academia and then to the general public. Finally it is important to remember that engineered nanomaterials, nanotechnologies and especially nanosafety are global issues that require global collaborations.

We have identified four clear priorities: 1) material characterization; 2) exposure, transport and life-cycle; 3) hazard mechanisms; and 4) risk assessment and management tools, but nonetheless there must be a continuous follow-up, review and evaluation to ensure the implementation of this proposed strategy.

In summary, this research roadmap aims at providing directions towards a sustainable development of nanotechnology based tools and products. It is based on the premises that a level of generalised knowledge in the different areas mentioned and dealt with above shall be achieved within the next 10-15 years and this will mean that the new materials coming onto the market will be safer by design and this philosophy will be beneficial not only for Europe, its citizens but for the whole world.
1 Introduction and background

The aim of this document is to introduce a strategic vision for future research to promote the safe use and application of engineered nanomaterials (ENM). The importance of this goal continues to increase due to the rapid expansion in the production of ENM and products incorporating them. This development will lead ultimately to mass production of a number of engineered nanomaterials, and thereby to increased exposure of workers and consumers to these novel materials. This development has also evoked increasing societal and public concerns on the safety of ENM and associated technologies. This evolution emphasizes the importance of setting priorities and goals on research of safety of ENM, thereby minimizing the uncertainties around the safety and health issues around these materials and nanotechnologies. Hence, the goal of the document in this context is also to describe the current level of knowledge of the safety of these materials and technologies, to identify knowledge gaps, and set out defined goals for the research on safety of ENM within the foreseeable future. Identifying research priorities assure that the set goals will also be reached. This will require the adoption of the aforementioned concreted goals with time-lines and milestones that enable the follow-up of the achievements of the proposed research priorities and goals to be identified later in this document. In this context, also the background and the current research environment and landscape need to be described. Key scientific observations and regulatory documents will also be referred to. The time horizon set in context will be set between 2015 and 2025, from the start of the first research project funded by the “EU Horizon 2020” Framework funding programme for research and innovation, until the end of the last project funded from that programme.

The European Commission has recently adopted a recommendation on the definition of nanomaterial (COM (2011) 696), according to which ‘nanomaterial’ means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution
1 Introduction and background

threshold of 50% may be replaced by a threshold between 1 and 50%. Even though this definition has been currently incorporated into some pieces of EU legislation, it has also recently been challenged because it has been considered to be an obstacle for research in addition to being a reflection of relatively a static, not a dynamic, understanding of the nature of nanosized materials for the purpose of regulations (Maynard, 2011). The definition has indeed been defended by the regulatory requirements of engineered nanomaterials (Stamm, 2011).

The potential of ENM and nanotechnologies to improve the quality of life and to contribute to economic growth and competitiveness of industry has been widely recognized, not only in Europe, but globally. Nanotechnologies can enable remarkable technological advances and innovations in many industry sectors. However, there is an ongoing scientific and political debate about potential risks of ENM and nanotechnologies (EU NAP, 2006-2009; NNE, Sep 2012; NNE Feb, 2013).

The EU 2020 strategy (COM(2010)2020 defines smart, sustainable and inclusive growth as the main European 2020 objective. Research and innovation have been identified as the key drivers of European social and economic prosperity, capable of promoting the achievement of environmental sustainability (EU Green Paper, 2011).

Competitiveness of the European industry is understood to be a priority in achieving these goals, and hence the role of innovations and an accelerated pace of the commercialization of innovations have been recognized as being fundamental in this respect. The recent Communication from the Commission on Horizon 2020 - The Programme for Research and Innovation (COM(2011)808) emphasizes the importance of research and innovation for society at large. The same issues have been stressed in the EU Nanotechnology Action Plan 2005-2009 (COM(2005)243). These strategic considerations all mean that there will be major changes in the future European research landscape and funding opportunities and all interested parties will need to adapt and prepare to meet these challenges. The proposal for establishing the new Programme for Research and Innovation - Horizon 2020 (COM(2011)809) places a major emphasis on securing a strong position on key enabling technologies (KET) such as information and communication technologies (ICT), nanotechnology, advanced materials, space technology or biotechnology, and underlines their significance for Europe’s competitiveness and its ability to provide the innovative goods and services essential for meeting global challenges. In particular, nanotechnology offers substantial possibilities for improving the competitive position of the EU and for responding to key societal challenges. Ensuring the safe development
and application of nanotechnologies has been included in the broad line of activities of Horizon 2020 proposal.

The new technology applications not only should be safe themselves but should also offer substantial improvements to human health and environment protection still remaining competitive. Due to the rapidly increasing production and use of ENM and utilization of nanotechnologies, safety aspects must be fully understood and addressed. In figure 1.1. a well protected researcher is performing carbon nanotube aerosol experiments.

According to a recent evaluation by the European Commission (2nd Regulatory Review of REACH (EC(2012) 572) it is unlikely that the size of nanomaterials per se causes hazards or harm to the human health or the environment. Small size does enable easy access to living organisms and hence may lead to increased risks to various living systems. This leads directly to scientific uncertainty about the general safety of these materials, stressing the need for safety assessment the nanosized substances. In fact, safety of ENM and nanotechnologies have been emphasized, not only by the European Commission and several EU Member States, but also outside Europe, e.g. in the US 2011 National Nanotechnology Initiative’s Environmental, Health, and Safety (EHS) Research Strategy (NNI, 2011) and in the recent report by US National Research Council,(NRC, 2012). It has been envisioned that the safety of processes as well as the technologies and products utilizing ENM, will be crucial in securing the success. Successful promotion and expansion of research on safety of
ENM need to be able to timely predict with accuracy and reliability the challenges and opportunities of novel ENM and nanotechnologies for the next 10 to 20 years, i.e. we need to aim at the year 2025. In a recent communication Nel et al. (2012) called for a totally novel testing strategies for ENM to enable the available human and other resources in order to cope with this ever increasing challenge. Earlier, Hartung (2009) has called for such an approach as well.

EU-level research funding from the framework programmes for research and the upcoming Horizon 2020 especially emphasizes the European added value, results which cannot be achieved by the actions or resources of a given EU Member State alone. The goal of this document is not only to provide a strategic level vision on research priorities on the safety of ENM, but also to illustrate how advances emanating from this research can be of benefit to the EU, its Member States, EU citizens, and globally. The time horizon of the document at hand - extended until 2025 - also allows the implementation and exploitation of results of nanosafety research projects funded from the Horizon 2020 - European Programme for Research and Innovation.

Towards this commitment, the European community of scientists addressing the safety of ENM and nanotechnologies in their research endeavour will establish coordination with EU and national authorities, industry and stakeholders. It will assist the collaboration between stakeholders and projects, particularly with national platforms and other industry platforms. The EU scientists dealing with research on safety of ENM are in many ways integrated in the European Technology Platforms such as NANO futures European Technology Platform for Industrial Safety (ETPIS), maintaining, however, their integrity and independence. The ultimate goal of the scientists addressing the safety of ENM in their research is to assure the safety of nanotechnologies from the handling of the nanomaterials, the manufacture of products incorporating, these materials to their safe use by the final user and their disposal i.e. safety throughout their entire life cycle. This will require establishing a new safety culture which should involve developing and implementing a complete system of methods, techniques and equipment and competent scientific and technical community, and to inform the general public about the safety management and governance of the technologies utilizing ENM.
Consequences for the vision for 2015-2025

Based on the achievements described above, the assessment of risks and their management will become routine among regulators. Industry will have adopted a way of working which will guarantee the incorporation of safety of the novel ENMs and nano-based products and processes, thereby markedly and positively contributing to the nanosafety in society within European Union and globally. This will require widespread adoption of the safe-by-design principle in the planning of ENM and in the creation of industrial processes utilizing these novel materials. The knowledge and awareness related to ENM and nanotechnologies among European citizens will have markedly improved i.e. citizens will be able to understand the fundamental issues surrounding nano-based products and issues important for ENM. This will have been due to neutral and reliable dissemination of information on ENM by regulators, academia, as well as the industry.

One of the key drivers of this positive development would be the creation of a positive industrial attitude towards nanosafety. It will have become self-evident to the different parties involved in the nanosciences associated with nanosafety that knowledge and trustworthiness are essential elements of success of nanotechnologies. Safety enables the creativity and new innovations and hence the ability of nanotechnologies to support wellbeing and social services. This positive development will allow regulators and decision makers to interact with other parties, academic community, industrial stakeholder, society at large, and various interest groups in the society. This is needed to carry out effective regulation of the safe use of ENM thus guaranteeing the safety of nanotechnologies and the products emerging from these processes.
1 Introduction and background

EU and international strategy documents

The importance of nanosafety has been emphasized in several documents and comments issued by the European Commission, several European Union Member States or other countries. The safety of ENM and nanotechnologies has been identified as one of the key drivers of the success of nanotechnologies in all of these documents. The European Union Green Paper (EU Green Paper, 2011) launched a public debate on the important issues to be taken into account for future EU research and innovation funding programmes. The document also emphasizes the importance of an empowering rather than a risk-driven approach in research on nanomaterials and nanotechnologies. In this context, the Green Paper consultation document notes the importance of strengthening of the European competitiveness, and it identifies key enabling technologies (KET) as means which would allow smart, sustainable and inclusive growth within the EU and beyond. Among the KETs, nanomaterials and nanotechnologies and their applications have a central position.

In its communication on Regulatory Aspects of Nanomaterials (COM (2008) 366) the Commission emphasizes that the current legislation in principle covers the potential health, safety and environmental risks in relation to ENM. However, the communication also notes the lack of knowledge of these issues on engineered nanomaterials and calls for more research and knowledge. Based on these goals and needs, later, in 2011, the Commission devised a definition for nanomaterials (COM (2011) 696). The importance of the safety of ENM and nanotechnologies had also been put forward in the Commission Communication on Nanosciences and Nanotechnologies: An Action Plan for Europe 2005-2009 (COM(2005)243) and subsequently in the Commission Communication on Nanosciences and Nanotechnologies: An Action Plan for Europe, Second Implementation Report 2007-2009 (COM(2009)697). This Implementation Report calls for European creativity, industrial innovations from knowledge to market, but it also addresses the societal dimension, i.e. expectations and concerns. It especially highlights health, safety, environment and consumer protection as well as bridging the knowledge gap between material sciences and applications of ENM to overcome the need of knowledge in areas like the characterization of ENM, toxicity, ecotoxicity, safety and exposure assessment. The implementation report also emphasizes the importance of international collaboration in
Introduction and background

The Commission has also aimed at promoting the implementation of safety of ENM and nanotechnologies in a number of Reach Implementation Projects. These include the Report of the European Commission’s Public Online Consultation: Towards a Strategic Nanotechnology Action Plan (SNAP) 2010-2015. Some of the key conclusions of the report were that more than 80% of the respondents have positive expectations toward nanotechnologies. ICT, energy, and the health care sector are seen especially promising areas of nanotechnology applications. There were also major concerns identified by the respondents. A key concern expressed by the industry was the rate of innovation in Europe and the risk that Europe may fall behind in the exploitation of its scientific base in nanotechnology. And finally, a concern shared by a number of parties was related to the safety of ENM and their regulation. Further details related to ENM were identified in several Reach Implementation Projects for example on substance identification (RIP-oN1) which included aspects about the applicability of REACH to ENM. The conclusion of this report, as that of many other reports, was that there are no specific definitions of ENM in REACH but that the general substance definition covers also ENM. As stated earlier, Commission Definition was launched by the Commission in 2011 (COM (2011) 696).

After the creation of the Commission definition on nanomaterials, little progress has taken place. The definition of nanomaterials will be utilized in the Cosmetics Legislation which will require labelling the presence of nanomaterials in cosmetics products which have been produced after July 1, 2013. Since there have been concerns about the slow development in the implementation of the definition in EU legislation, the Governments of Austria, Belgium, Czech Republic, Denmark, France, Italy, Luxembourg, Spain, Sweden, Croatia and the Netherlands have called for action from the Commission and urged that it to undertake actions to guarantee the health of European citizens and the protection of the environment by ensuring that EU legislation takes possible risks associated with nanomaterials into account. A note from the Dutch Government, supported by the Governments of the other aforementioned EU Member States urged that the European Commission should take the following measures without delay “: 

relation to nanotechnologies, ENM, nanosciences and nanosafety in the context of OECD, ISO and CEN.
1. Provide for adaptations to existing legislation (e.g. on chemicals, biocides, cosmetics, additives and labelling) to improve the application of nanomaterials;

2) Propose legislation on registration or market surveillance of nanomaterials or products containing nanomaterials;

3) Either through an amendment of REACH or through supplementary legislation, whatever is most appropriate given the urgency, publish a proposal or an appropriate mix of effective measure that should include:
   - a solution to the current lack of definitions within REACH;
   - a review of the current tonnage levels for nanomaterials within REACH;
   - shortening the period within which information must be obtained;
   - introducing specific requirements for nanomaterials such as characterization and testing”.

Two recent and important United States Strategy documents have discussed issues related to the safety of ENM and societal needs associated with them. The report by Roco et al. (2012) “Nanotechnology Research Directions for Societal Needs in 2020” discusses four major topics:

1) methods and tools of nanotechnology for investigation, synthesis, and manufacturing;

2) safe and sustainable development of nanotechnology for responsible and effective management of its potential including EHS aspects and support for a sustainable environment in terms of energy, water, food, raw materials and climate;

3) nanotechnology applications for advances of bio-systems and medicines, information technology and other technology areas;

4) societal dimensions including elucidation, investing in physical infrastructure, and governance of nanotechnology for societal benefit.

The report describes in a great detail progress in these areas since 2000. The report constantly emphasizes the importance of the safe and sustainable development of nanomaterials and nanotechnology applications. The main conclusions of the report can be summarized as follows:

1) continuous support for basic nanotechnology research;

2) promoting focused R & D programs;

3) advancing partnerships between industry, academia, NGOs, multiple agencies and international organizations;
4) support of precompetitive R & D platforms; 5) global coordination of nanosciences;
6) develop experimental and predictive methods for exposure and toxicity to multiple nanostructured compounds. In addition, the report emphasizes 7) horizontal, vertical, and system integration nanotechnology education to create or expand regional centres for learning and research; and 8) exploring new strategies for mass dissemination, public awareness, and participation related to nanotechnology R & D breaking through numerous barriers.

The take-home message of the report is that a wide perspective on nanotechnologies including safety and societal needs is an absolute necessity for the future success of nanotechnologies.

The National Nanotechnology Initiative has published recently (NNI, 2011) a strategy document with a focus on human health, safety and the environment. This provides the US Federal Government’s view on key strategy issues of EHS in the US. It also sets strategic goals for nanotechnology related EHS research and identifies the means to reach the goals set for 2020.

**Elements of the strategy**

Governmental funding agencies globally, European Union, and private funding agencies have recently invested remarkable amount of resources to support the research on safety of nanomaterials. For example, this is clearly reflected in the numbers of scientific publications during last decade which have increased in an exponential fashion. There has been a rapid increase in the publications on human health since 2000, less rapid on ecotoxicology since 2005, but the number of papers on exposure and assessment of exposure has remained low throughout this period (Figure 1.2.).
In addition, the number of breakthroughs and remarkable innovations has been much smaller than expected in spite of the markedly increased volume of nanosafety research and its funding. This has prompted the funding agencies, governments and the academic community to search for ways to improve the cost benefit ratio of the research undertakings, and to emphasize the social dimension and societal needs of this research.

From the societal point, research serving risk assessment, management and governance has been modest in terms of usefulness, timeliness and its systematic nature. Much of the research has focused on mechanistic issues which may have remarkable merits but do not serve well the urgent societal needs. At the same one has to appreciate the concept that mechanistic innovations may well be of immense importance in the future also from regulatory perspective, since the regulations need to be based on scientific research.

The number of strategy documents aiming at providing direction of re-

Figure 1.2. depicts the total number of papers published during years 2001-2009 in the open literature on EHS of engineered nanomaterials, and separates papers on exposure assessment, ecotoxicology and human toxicology.
search on the safety of ENM and nanotechnologies EU Action Plan 2006-2009 (NAP 2006-2009), NNI Strategy document (NNI, 2011) and EU Green paper (2011) as well as the NAS EHS Strategy (2012) document seeking to find ways to identify a set of priorities has rapidly increased. They all aim at finding ways to serve societal needs to assure the safety of ENM. In these documents, also the concept has been made that the limited resources need to be considered and that duplication of research efforts needs to be avoided.

The key elements of the strategies usually include the following important items: 1. Description of the current state of knowledge, the existing research landscape and identification of the requirements of the research environments and infrastructures essential for the promotion of research on safe ENM and nanotechnologies. 2. Identification of societal needs for the regulation of safety of these materials and technologies. 3. Identification of the necessary research goals for fulfilling of the societal needs and setting a time-line with milestones for the follow-up of the progress of the research endeavors. 4. Identification of the research priorities that allow reaching the goals within the time-limit set by the strategy. 5. Identification of the means by which the results of the research can be disseminated, implemented and exploited to evoke a change in the ways industry can promote safe use of ENM and assure the safety of workers and consumers, enabling regulators to make educated regulatory decisions. 6. Identification of the needs for further possible regulatory actions and possible further investments into infrastructures, educational and funding programs to be able to fully utilize the technological and economic benefits of these materials and technologies. Many of these elements are general for any given research strategies, but when dealing with new and emerging technologies, public perception is always at the heart of the strategic activities and has to be carefully considered. All these considerations highlight the importance of understanding of the associated research landscape.

**Towards understanding of nanomaterials**

ENM are characterized by their complexity. This complexity is evident in not only in their physico-chemical characteristics and behaviour but also in their interactions with living systems. In some respects, they can be viewed as mater-
Nanotechnologies and nanomaterials, in addition to being a new material paradigm, are at the same time positioned at the interface of many scientific disciplines. Hence, nanotechnologies utilizing nanomaterials are in a unique position to nurture novel innovations. Due to the diverse nature of nanomaterials, they have contributed to the birth of several subfields of nanosciences such as optoelectronics and printed electronics, innovative construction materials, novel surfaces and packaging. They have been the impetus for the creation of the discipline of safety-related nanotoxicology which is the foundation of the safety assessment of ENM, the focus of this document.

Nanosciences and -technologies deal with the manipulation of matter in a size-range from 1 to 100 nm in at least one dimension. Some of the recognized engineered nanomaterials have dimensions even smaller than 1 nm (e.g. fullerenes). In figure 1.4. some familiar objects are shown in a nanometer scale for comparison. This and the more narrow EU definition do not, however, reveal the truly innovative nature of nanotechnology and the nanosciences, a criticism leveled by Maynard (2011). Indeed, the study of nanotechnologies and nanosciences demands creativity because at the nano-scale particles have the potential to interact with living organisms at the cellular even at the molecular levels (see Monopoli et al., 2012). In essence, cells are a biological micro-cosmos, the elements of which are expressed at a nanoscale thereby bringing a qualitatively novel dimension to the material-biology interplay (Shvedova et al., 2010).

Figure 1.4. Size of objects in a nanometer scale. (modified from: National Cancer Institute, USA).
When one considers the characteristics of materials at the nanoscale, then the diversity of classes and subgroups of nanomaterials is truly astonishing. Examples of different and emerging types of nanomaterials include functionalised carbon/organic/metal nanotubes, nanowires, metal, metal oxide and organics nanoparticles, and graphene (Figure 1.5). They also include bioactive and biodegradable ceramics and polymers, active gels, piezoelectrics, electrostrictives, ferroelectrics, multiferroics, nonlinear/tunable metamaterials, shape memory materials, supramolecular polymers, stimuli sensitive polymers, and possible combinations of these materials. Sometimes they are present in organized arrays, and sometimes as simple mixtures. They can express characteristics that enable self-assembly of nanosized materials into chemically similar but micro-sized structures, one example being nanocellulose. However, it is the flexibility for manipulation which endows all these materials with a world of possibilities and this is why products with nanomaterials can be so creative and innovative.

However, this plethora of different types of materials at nano-scale not only confers almost unlimited technological benefits but it also holds the possibilities for unexpected interactions between these materials and biological systems at the molecular, cellular, organ and organism levels (see Nel et al., 2012; Shedova et al., 2010).
Nanotechnology and ENM already have had a major impact on electronics, coatings, construction, food technology, the design of new materials such as nanocellulose applications, telecommunication, environmental technologies, medical technologies and drug development, nano-biocide applications and energy production as well as new agriculture, water purification systems and the utilization of solar energy among others. The possibilities associated with ENM are seemingly unlimited, and the developments seem to be proceeding towards ever more complexity. This also poses challenges for the regulation and societal control of unlimited utilization of these possibilities. In addition to their impact on society, also the possible effects of ENM on human health and consequences to the environment are now recognized as very important. Public awareness and concern on the potential hazards of the applications of these materials and technologies are consequently on the rise, and these need to be taken into account, not ignored.

Hierarchy will be a key issue in the future development of nanotechnologies. Hierarchy refers to the control of structures in space on a nanoscale. However, it also means control of the evolution of a material with time. Hierarchy implies combining, in a predictable way, several nanomaterials, and finding synergies between them. But nanotechnology offers also the possibility to explore multiple combinations and their subsequent consequences at the nanoscale; these novel material characteristics can be thus anticipated or totally unexpected, positive or negative involving material-biology interactions. Weak interactions such as hydrogen bonding or coordination interactions may play a crucial role in the evolution of the various events possibly occurring at the nano-scale, and which may play a crucial role in the evolution or in the generation of man-made nano-scale applications.

Importance of nanosafety

Nanotechnologies belong to emerging technologies which hold the promise of bringing significant economic and technological benefits but for which there is only limited knowledge on their possible hazard potential; for example exposure to these materials either in the environment or via consumer products. Assessing true workplace exposure may be challenging in some cases because of lack of understanding of the predictive value of different metrics in predicting human hazard and risk. Today, the most urgent challenge related to
ENM and nanotechnologies is how can we gather the essential knowledge that could be utilized for reliable risk assessment and adequate risk management and governance. Even though there is increasing amount of information of the hazard potential on several ENM, there is a dramatic lack of systematic, and especially relevant, information on the potential hazards associated with these materials. What is lacking is the kind of scientific knowledge which would be suitable for regulatory decision making i.e. reliable risk assessment data. As valuable as mechanistic studies are for our understanding of the potential hazard mechanisms of these materials, using their results in regulatory risk assessment is challenging unless they are associated with acceptable experimental animal studies serving risk assessment and management purposes.

To be able to respond to the societal needs for safe nanomaterials and nanotechnologies, it is necessary to complement valuable mechanistic studies with systematic short-term and long-term animal experiments that would allow a reliable estimation of the possible risks of ENM, e.g. to provide the predictable no-effect levels (DNEL) which is a value widely used in the risk assessment of engineered nanomaterials and other chemicals in the context of REACH legislation. Currently, studies on nanomaterials cannot be used for risk assessment unless they have been adequately validated against appropriate animal studies as this is the only way to demonstrate that they have any predictive power. At present, these kinds of validated in vitro studies simply do not exist, and this has hindered the development of novel intelligent testing strategies of these materials.

The current situation is especially challenging, as testing abilities and resources do not allow investigators to make an adequate assessment of safety and risks of ENM (Environmental Science and Technology, 2009). Hartung (2009) in his paper "Toxicology of the 21st Century" published in Nature called for abandoning the existing experimental animal testing paradigm and moving to the use of high-throughput testing approaches. This is a very positive proposal which has been supported by Nel et al. (2012) who also emphasized the use of in vitro approaches utilizing high-throughput technologies and omics methodologies in the safety and toxicity testing of ENM. From the regulatory point of view, these proposals while being very attractive, they have one critical flaw; but both lack the fundamental justification for their adoption. As long as there is no clear evidence of the predictive power of these approaches for in vivo systems, their acceptance as a part of the regulatory framework of nanomaterials or other chemicals is highly unlikely; there are simply too many uncertainties and safety is not an area where we can be afford to take risks with untested technologies and techniques. Figure 1.6. shows an example of mouse lung tissue after
exposure to carbon nanotubes.

Currently most of the existing \textit{in vitro} or \textit{in vivo} toxicity studies of ENM allow - at best - one to make some kind of comparison in terms of the relative hazard potential provided that the batch-to-batch variability has been considered, the materials adequately characterized, the appropriate number of graded doses have been used, and predictive endpoints have been measured. Often the data are interesting but not suitable for quantitative or even qualitative risk assessment. In addition to the shortcomings described above, very little is known of the true internal dose of the materials to which living organisms are being exposed. In most cases we do not have adequate methods to assess many crucial parameters e.g. the dose received by liver or other organs, because we do not possess the required knowledge of the translocation of the materials in the body and across biological barriers, or the means to assess the dose in a given organ or type of cell \textit{in vivo}. (Oberdörster et al., 2005).

All the above considerations are highly relevant when assessing the safety of ENM. One of the major challenges is that, in many cases, technologies that would deliver the required piece of information do not exist, or even if theoretically the techniques are available, they would require such exhaustive resources to negate their implantation. There is enough information to question whether all the assessments are necessary - or, in the worst case scenario - enough. The majority of ENM may be harmless or only modestly harmful, but there is a plethora of evidence revealing many of the materials may be highly harmful. Hence, the crucial challenge in all cases is to identify the harmful agents and to differentiate them from their innocent counterparts so that the appropriate regulatory decisions can be made to protect human health and the environment.
Thus, it is very important to gather reliable data on toxicity of ENM to be used for risk assessment. It is equally important to gather all available data on exposure levels in workplaces, in the environment and through consumer products so that the state-of-the-art in terms of hazard and exposure can be evaluated (Figure 1.7.). These data can be used for further development of regulatory decisions and risk management and governance of ENM. These activities are currently being carried out within the frame of EU 7th Framework Programme (FP7) projects such as MARINA, NanoValid, and NanoRegulatory Testing, all currently running large EU FP7 funded IP Projects (NSC Compendium, 2013). The major investments do not, however, resolve the underlying challenge in the assessment safety of ENM and nanotechnologies, notably the gap between available resources and safety/toxicity assessment needs (Hartung, 2009; Nel et al., 2012). Most, if not all, of the current nanosafety research projects deal with the so called first generation passive nanomaterials, and the present challenges are truly immense. However, soon markets will be flooded with much more complex 2nd and 3rd generation nanomaterials, and research on these materials is in its infancy and some investigators have stated that we do not possess the necessary research tools to evaluate their effects (see Roco et al., 2010).

At the same time as data starts to become available and improving our understanding of the potential hazards and exposure to the first generation nanomaterials, there is an urgent need to develop totally novel paradigms for the testing of the second and third generation materials (Roco et al., 2010). This will require a systematic initiative to investigate the causalities and associations between characteristics, and the interactions of ENM with biological systems at molecular, cellular, organ and organism levels, without forgetting various relevant disease models. These studies should aim at identifying those material characteristics that are associated with a harmful endpoint at some of these levels. Bioinformatics and systems biology approaches could be highly advantageous in the development of these new safety/toxicity assessment paradigms, which could allow us to make an evaluation of the risks associated with various classes of ENM. These systematic undertakings should cover the entire life-cycle of various ENM from cradle to grave. For example, an assessment of leakages of various nanomaterial incorporating products will be imperative if we are to be able to assess the potential exposure of humans and the environment to these materials. However, as long as safety aspects are ignored in the design of novel ENM, and nano-enabled products or processes, the assessment of safety of nanotechnologies will remain a major issue and a tremendous burden to the industry.
Hence, in the coming years, a remarkable challenge for the nanotechnology industry, the academia and the regulators will be the exploitation a novel safety culture to the nanomaterial research and engineering community. Incorporating the safety-by-design as a part of the core research activities of nanomaterial sciences and the production of ENM would be a major step forward in assuring nanosafety. This strategy will not be inexpensive, but the benefits that it will confer will be undeniable. In order to incorporate nanosafety as part of the creative process and in the final formulation of the devices or materials themselves, some steps must be taken.

As mentioned above, it is first necessary to gather knowledge on the safety issues of ENM e.g. relating toxicity of the materials with material characteristics, to learn about the toxicity of the different generations of materials, their stability and degradability. Such information is available for a small number of ENM, but for most materials this information is lacking. Then, a proper risk assessment throughout the Life Cycle of the materials from their generation to disposal becomes an unavoidable issue. The Life Cycle Analysis needs to become a key tool for safety assessment. It will be necessary to know when and where the product throughout its lifetime could be viewed as hazardous. The potential for exposure must be assessed separately for each of the generations of nanomaterial to the end of their life-cycle. In the risk analysis, the integration of a nanomaterial into some material, and the use and possible degradation of the materials must be taken into account.

This knowledge must be then integrated in the fabrication process but the success of this approach will only become apparent when scientists, government, and industry are convinced that this is the way to proceed. A strong dissemination and exploitation of this new approach is therefore required to create the awareness-consciousness of the various benefits and risks and ways that risk can be mitigated in the context of nanotechnologies. In addition, tools must be provided to ensure the transfer the knowledge acquired in the nanosafety studies done in research institutes to the companies manufacturing nanomaterials. There must be standardized procedures to deal with nanomaterials and products entailing ENM. Finally, the expected massive production of ENM may lead to new issues related to their disposal and the treatment of ENM waste. This is an aspect which is too easily overlooked but it has to be recognized that it may have important long-term consequences for the human health and the environment. Basic questions such as should ENM be disposed as such or should they be processed prior to their disposal still need to be addressed.
Figure 1.7. It is important to study exposure levels and evaluate the effects of nanomaterials both in workplaces and in the environment; a) Light microscopy picture of lung tissue with carbon nanotubes inside the cells (photo by Esa Vanhala, FIOH); b) Light microscopy picture of Daphnia magna with the gut full of algae (photo by Kukka Pakarinen, UEF); c) Workers handling nanomaterials are well protected against exposure (photo by Emmi Kallio, copyright FIOH)
Conclusions

The challenges associated with the safety of nanomaterials and nanotechnologies are global. The commercial benefits may be outweighed by the potentially harmful characteristics of ENM. Since these emerging materials and technologies have been identified as the key enabling technologies guaranteeing the ability of key-industrial sectors to compete globally, their success requires also demands an assurance of their safety.

Today’s key challenges are that available tools for the assessment of the safety are often inappropriate, or so laborious that adequate risk assessment of these materials and technologies remains highly problematic. Current resources or testing tools are not likely to enable safety assessment of the novel ENM that are flooding the markets. This means that totally new safety assessment paradigms need to be developed during the coming years to solve this problem. At the same time it is important to maintain the level of current resources to assess the safety of ENM and to support the regulators and industry so that it can maintain its ability to provide the prosperity and wellbeing of citizens within Europe and beyond. This current situation calls for rapid identification of research priorities and development of roadmap for necessary undertaking to assure the safety of nanomaterials and nanotechnologies in the future. Unfortunately this endeavour cannot be undertaken without a clear understanding of the current research landscape within Europe and beyond and the analysis of the environment with the societal challenges defining why these activities need to be carried out. The research landscape will be tackled in the following chapter. The aims are to provide an understanding of the key challenges which we must confront in our attempts to promote the safety of ENM and nanotechnologies. The best ways to conduct research on nanosafety to ultimately confirm their success are presented in Figure 1.8. Because of the nature of research and the time gap between discoveries made in the laboratory until their implementation and exploitation; we have decided on a time
horizon from 2015 until 2025, i.e. the years that are relevant for the execution of the “Horizon 2020” the next EU Funding Programme for joint European Research and Innovations.

Nanosafety for Innovation and Sustainability

Figure 1.8. Key challenges in dealing with in order to promote the safety of engineered nanomaterials and nanotechnologies
References:


1 Introduction and background

NSF Strategy 2011,


RIP-oN1; Reach Implementation Projects for example on substance identification


Stamm, H. Nanomaterials should be defined. NATURE vol. 476 no. 7361 p. 399.
2 The nano-safety research landscape

Nanosafety: facing the challenges

Nanotechnology is hailed as one of the enabling technologies to innovation. Nanosafety, in turn, is concerned with the safe and sustainable development of nanotechnology. Without nanosafety research, widespread use of nanotechnologies in many sectors of society would be hampered and could even come to a complete stand still.

"Nanotechnology" was first introduced in 1971 by Norio Taniguchi as a term for ultra-precision machining. Nanosafety research, on the other hand, is a relatively new discipline. When the Royal Society and the Royal Academy of Engineering, UK, report was published in 2004 on the implications of nanoscience and nanotechnologies, very little information was available on how exactly engineered nanomaterials interact with biological systems. Indeed, although humans have been exposed to airborne nano-sized particles throughout evolution, such exposure has increased dramatically over the last century due to anthropogenic sources. Moreover, as stated by Oberdörster et al. (2005) in their landmark review on the emerging discipline of nanotoxicology, the rapidly developing field of nanotechnology is likely to dramatically increase the exposure to nanomaterials through inhalation, ingestion, skin uptake, and injection (for clinical applications). Results of previous work on particles and fibers (man-made or natural) can be viewed as the basis for the expanding field of nanotoxicology, which can be defined as safety
evaluation of engineered nanostructures and nanodevices. Moreover, it is the very fact that man-made nanomaterials may interfere with biological systems at the nano-scale that raises cause for concern (Shvedova et al. 2010).

Figure 2.1. Expected size of the market of products incorporating ENM by 2020. Adapted from Roco et al (2010).

It is important to consider that we are still dealing only with first generation nano-enabled products (i.e. passive nanostructures) but it is likely that we will move soon to second generation products containing active nanostructures, to third generation systems of integrated nano-systems and, finally, by the year 2020 according to some predictions, to fourth generation products or heterogeneous molecular nano-systems that allow molecular devices ‘by design’ (see Roco et al., 2010). This means that methods for assessment of the safety of next generation nano-enabled products also must evolve: nanotechnology is a moving target and toxicologists cannot afford to miss the train.
The challenges that we are facing in nanotoxicology are not unique to this sub-speciality of toxicology but there is an urgent need for a “new” toxicology, toxicology for the 21st century. A proposal for a new, systems biology / toxicology approach was put forward in a 2007 report by the US National Academy of Sciences on behalf of the US Environmental Protection Agency (EPA). The overall aim is to enable a shift from toxicity testing primarily in animal models to in vitro assays and in vivo assays using lower model organisms, along with computational modeling, thus enabling the evolution of toxicology from observational science to a predictive science (Collins, 2008). The central part of this novel toxicology is to describe toxicity pathways which lead to molecular understanding. This allows the development of novel predictive models using computational approaches. In 2007, the US EPA launched a project entitled ToxCast to predict toxicity of chemicals using computational, high-throughput screening (HTS) approach, and various toxicogenomic technologies, in line with the call for a new approach to chemicals testing. Hartung (2009) has also argued that “the testing of substances for adverse effects on humans and the environment needs a radical overhaul if we are to meet the challenges of ensuring health and safety.” In fact, he provocatively stated that “there is almost no other scientific field in which the core experimental protocols have remained nearly unchanged for more than 40 years” and proposes that an entirely new system is urgently needed and should be built from scratch, using modern methods. It may be prudent to note that an evolving scientific discipline such as nanotoxicology is optimally positioned to take on board these new approaches.

Figure 2.2.
A macrophage full of nanosized titanium dioxide (black dots).
(Photoby Esa Vanhala, FIOH)
The large EU FP7 projects, NANOSOLUTIONS and NANOMILE are both dedicated to systems biology approaches to understanding the interactions of engineered nanomaterials with living organisms and the environment.

The European Commission has invested a considerable sum in nanosafety-related research projects: close to fifty projects are either completed or ongoing and represent a total RTD investment of €137 million from the NMP and other programmes, with 13 projects (€31 million) under FP6 and 34 projects (€106 million) to date under FP7. These projects represent a significant effort of the scientific and industrial research community in Europe. A considerable amount of data on the potential hazard of ENMs has accrued while information on exposure to ENMs and on safety of nano-enabled products throughout their life cycle is still lacking. The problem with the hazard data obtained to date is that the results do not allow for any general conclusions. This is, in part, due to the lack of standardized methods and reference materials for toxicity assessment (Krug, 2011). In an attempt to resolve some of these issues, the European Commission has recently funded two large FP7 projects (MARINA and NanoValid) devoted to the development and validation of reference methods and materials for life cycle analysis (LCA), exposure, hazard identification, and risk assessment of ENMs. The two projects have a combined budget of €18.6 million and will run for 4 years (2011-2015).

We still lack a fundamental understanding of how nanomaterials interact with living systems and, thus, we are not yet in a position to assess the relevant end-points for nanomaterial toxicity. At the same time, we are faced with an onslaught of new materials for which testing or screening of toxicity is required. To resolve this situation, methods for prediction of nanomaterial toxicity are needed.
Action plans and strategic funding instruments


Following the Action Plan, the Second Implementation Report 2007-2009 stated: nano-enabled products will enjoy public acceptance only if regulations adequately address the new challenges from the nanotechnologies, if manufacturers can demonstrate their safety, and if consumers perceive them as safe. This remains the key challenge today and into the foreseeable future, as the number of potential applications for nano-enabled products continues to increase.

More recently, the European Commission held the public consultation “Towards a Strategic Nanotechnology Action Plan (SNAP) 2010-2015”. The European Commission is thus considering a new Action Plan for Nanotechnology, addressing the technological and societal challenges and strengthening the research and innovation efforts, with increased emphasis on sustainable development, competitiveness, environmental, health, and safety (EHS) issues. The latter is of great relevance for the present document: there is a growing realization that EHS issues are of crucial importance for the successful implementation of nanotechnologies.

Figure 2.3 Generator to aerosolize carbon nanotubes. (Photo by Joonas Koivisto, FIOH).
In addition, several European Member States present highly elaborated nanotechnology research portfolios. For instance, in 2011, the German Federal Ministry for Education and Research announced the adoption of a new Nanotechnology Action Plan 2015, carrying on from the Action Plan of 2010. Nano-safety research benefits from a close dialogue between the different actors – not only academic institutions but also industry - and the European NanoSafety Cluster provides a forum for such interactions.

Looking beyond Europe, the National Nanotechnology Initiative (NNI) in the United States coordinates funding for nanotechnology research and development among the 26 participating federal departments and agencies (see: www.nanogov.org). The 2013 US Federal Budget provides $1.8 billion for the NNI and the cumulative investment since 2001 totals over $18 billion and cumulative investments in nanotechnology-related EHS research since 2005 is in total $650 million. In 2008, two Centers for the Environmental Implications of Nanotechnology (CEIN) were established with the focus on the assessment of nanomaterial interactions with the environment and with living systems.

While joint calls for research proposals have proven difficult to implement, US and European scientists have nevertheless found ways to cooperate in the field of nanosafety. In the FP7 project NANOMMUNE, devoted to studies of ENM effects on the immune system, almost half of the participating principal investigators were from the United States. In a more current example, the FP7-NanoTransKinetics project is paired with the US project “Nanoparticle Transport: From Cells to Organisms” funded by the US EPA, thus enabling EU and US scientists to work towards common research goals. However, these are scattered examples and more concerted efforts are needed; see below on international cooperation.

An ERA-NET is a coordination activity funded by the European Commission in FP7. The main objective is to provide a framework to network national and regional research programs, leading to concrete cooperation such as development and implementation of joint transnational calls for proposals. The FP7 ERA-NET on Nanosafety: Safe Implementation of Innovative Nanosciences and Nanotechnology (SIINN) started in 2011 and it will run for 3 years. The activities aim to promote the safe and rapid transfer of European research results in nanoscience into industrial applications. National and regional resources are virtually pooled to create a transnational programme of research.
The first call for transnational research proposals focused on the following topics:

- models and methods for analytical tools, theoretical prediction, and characterization
- exposure assessment
- impacts of nanomaterials on the environment
- properties and effects of ENM

COST is an inter-governmental framework for European Cooperation in Science and Technology, allowing the coordination of nationally-funded research on a European level. The key features are:

- building capacity by connecting high-quality scientific communities throughout Europe and worldwide
- providing networking opportunities for early career investigators
- increasing the impact of research on policy makers, regulatory bodies and national decision makers as well as the private sector

Through its inclusiveness COST supports integration of research communities, leverages national research investments and addresses issues of global relevance. In 2012, the COST action MODENA (“Modeling Nanomaterial Toxicity”) on Quantitative Nanostructure-Toxicity Relationships (QNTR) computational modelling was approved and launched. QNTR computational modelling is an effective alternative to experimental testing since it enables the prediction of (eco)-toxicological effects based on ENM structure only (Puzyn, 2012).

There are a number of instruments for funding and coordination of nanosafety research at the EU level and at Member State level. Nevertheless, in order to maximize the benefits of such investments, more concerted efforts are needed. The European NanoSafety Cluster may facilitate such interactions.
European NanoSafety Cluster

The European NanoSafety Cluster is a DG RTD NMP CSA initiative to maximize the synergies between the existing FP6 and FP7 projects addressing all aspects of nanosafety including materials, hazard, databases, modeling and dissemination (Figure 2.4.). Synergy among the various FP6 and FP7 projects on nanosafety and other national projects, collaboration for maximizing impact, policy elaboration, planning of future actions, and international cooperation are the main aims of the European NanoSafety Cluster. About fifty projects dealing with nanosafety have either been completed or are running under FP6 and FP7. These projects together with a significant number of nationally supported projects represent valuable efforts of the scientific and industrial research community towards progress. Information on all current or recently completed projects is collected in the NanoSafety Cluster Compendium (www.nanosafetycluster.eu).

**The main objectives of the European NanoSafety Cluster are to:**

- facilitate the formation of a consensus on nanotoxicology in Europe
- provide a single voice for discussions with external bodies
- avoid duplicating work and improve efficiency
- improve the coherence of nanotoxicology studies and harmonize methods
- provide a forum for discussion, problem solving and planning R&D activities in Europe
- provide industrial stakeholders and the general public knowledge on the risks of nanomaterials for human health and the environment

Synergy among the projects, collaboration for maximizing impact, and international cooperation are the main aims of the European NanoSafety Cluster.
2 The nanosafety research landscape

Figure 2.4. Organization of the European NanoSafety Cluster
European stakeholder collaboration platforms

The European Technology Platforms

The European Technology Platforms (ETPs) were launched some 10 years ago as industry-led stakeholder forums to define medium to long-term technological objectives and develop roadmaps to achieve them. Their aim was to contribute to increasing synergies between different actors, ultimately enhancing European competitiveness. The ETPs are independent organisations whose creation was supported by the European Commission. All ETPs have brought together stakeholders, reached consensus on a common vision and established a Strategic Research Agenda (SRA). Some have also developed a plan detailing the actions required to implement the SRA.

There are several ETPs of direct relevance to the nanosafety research community, including the ETP on nanomedicine, ENIAC (nanoelectronics), SusChem (sustainable chemistry), ETPIS (industrial safety), and the ETP on innovative medicine. The European Technology Platform for Industrial Safety (ETPIS) aims at the improvement of industrial safety by co-ordinated efforts in research, improved risk assessment and control methodologies. The vision for industrial safety performance as stated in the Strategic Research Agenda (2006) is summarized as follows:

By 2020 in European industry
- a new safety paradigm will have been widely adopted. Safety is seen as a key factor for successful business and an inherent element of business performance.
- a structured self-regulated safety program is available. This will have firm, measurable performance targets for improved structural performance, accident elimination and will meet the annual reduction rate stated in the Technology Platform objectives
- accident-free workplaces will become the norm. This development will significantly contribute to the sustainable growth by safer utilization of technologies and life extension of ageing structures and, hence, with the improvement of social welfare
ETPIS is organized to nine Focus Groups and Hubs, one of which is for Nanosafety. The overall objective of the HUB NANOSAFE is to develop synergies between projects dealing with safe nano manufacturing. This includes the development of:

- advanced detection and monitoring technologies at workplace
- secure integrated industrial processes
- a global approach all along the life cycle
- knowledge on health and environmental effects of nanoparticles

Clearly, all of these strategic aims are aligned with the aims and visions of the European NanoSafety Cluster and its member projects. Thus, close cooperation between the ETPIS and the nanosafety projects is needed. The NANOfutures platform provides one such forum for integration (see below).

**NANOfutures**

The FP7 project, NANOfutures (www.nanofutures.eu) is a European Technology Integrating and Innovation Platform (ETIP), a multi-sectorial, integrating platform with the objective of connecting and establishing cooperation and representation of ETPs that involve nanotechnologies in their industrial sector and products. NANOfutures is open to industry, SMEs, NGOs, financial institutions, research institutions, universities and civil society. It is an environment where these different entities are able to interact and develop a shared vision on nanotechnology. NANOfutures collaborates with ETPs on the basis of a Memorandum of Understanding. Eleven ETPs from different industry sectors participate in NANOfutures.

The recent NANOfutures Roadmap (2012) addresses key issues related to cross-sectorial research, technology and innovation as well as broad socio-economic challenges to the implementation and commercialisation of sustainable and safe nanotechnology. NANOfutures seeks to address why the economic and societal benefits from the major investments in nanotechnology research have not, as yet, materialized. There are probably several explanations for this, including the fact that broad cross-sectorial issues, including safety and regulation, have not been addressed in sufficient detail. The present document takes into account the work that is being carried out in NANOfutures but aims to define research priorities specifically for nanosafety.
International cooperation, standardization and education

Research on the impact of the increasingly widespread nanotechnologies on human health and the environment is of global concern. It is important to avoid duplication and to leverage resources at the international level, not least in the field of nanosafety. European researchers are engaged in a dialogue with their US counterparts in the field of environmental, health, and safety (EHS).

The US-EU dialogue, “bridging nano-EHS research”, has three goals:

1. to engage in an active discussion on nano-EHS issues
2. to encourage joint programs of research
3. to establish communities of practice between researchers and corresponding funding sources to enable collaborations

Communities of Research (CoRs) are formed by groups of people, sharing a significant interest in the field of nanosafety. CoRs develop a shared repertoire of resources: experiences, tools, ways of addressing recurring questions and challenges (for further information, see: www.us-eu.org). The first three CoRs (predictive modelling for human health; ecotoxicity testing and predictive models; exposure through the life cycle) are supported from the National Nanotechnology Coordination Office in the United States while the remaining three CoRs (databases and ontology; risk assessment; risk management and control) receive administrative support from the European Commission. Each CoR has one EU co-chairperson and one US co-chairperson. The dialogue is promoted through regular meetings (Washington, DC in 2010; Helsinki, Finland in 2012) and in addition, each CoR organizes webinars, telephone meetings.
The following Communities of Research (CoRs) in nanosafety have been launched:

- Predictive Modeling for Human Health
- Ecotoxicity Testing & Predictive Models
- Exposure Through the Life Cycle
- Databases & Ontologies
- Risk Assessment
- Risk Management & Control

The OECD established the **Working Party on the Safety of Manufactured Nanomaterials (WPMN)** in 2006 with the objective to promote international cooperation in human health and environmental safety related aspects of manufactured nanomaterials in order to assist in the development of rigorous safety evaluation of nanomaterials. The WPMN brings together more than 100 experts from governments and other stakeholders from OECD countries and non-member economies. The work is implemented through specific projects to further develop appropriate methods and strategies to help ensure human health and environmental safety:

- OECD Database on Manufactured Nanomaterials to Inform and Analyse EHS Research Activities
- Safety Testing of a Representative Set of Manufactured Nanomaterials
- Manufactured Nanomaterials and Test Guidelines
- Co-operation on Voluntary Schemes and Regulatory Programmes
- Co-operation on Risk Assessment
- The role of Alternative Methods in Nanotoxicology
- Exposure Measurement and Exposure Mitigation
- Environmentally Sustainable Use of Manufactured Nanomaterials

The Sponsorship Programme on the Testing on Manufactured Nanomaterials was launched in 2007. The program involves the pooling of expertise in OECD countries and non-member economies and funding of the safety testing of specific manufactured nanomaterials according to an agreed upon priority list and a list of end-points relevant for human health and environmental safety (www.oecd.org). The OECD publication series on the safety of manufactured nanomaterials provides a wealth of information including guidance manuals for safety assessment of nanomaterials.
The International (ISO) and the European (CEN) standardisation bodies have established working groups on nanotechnology. These working groups also cover the nomenclature and metrology of nanotechnology and, hence, the issue of definitions of nanomaterials. The definition of nanomaterials has been the subject of heated debate and the Joint Research Centre (JRC) of the European Commission recently published a report on “Considerations on a Definition of Nanomaterial for Regulatory Purposes” (2010). The report gives an overview of definitions by international, national and European institutions, and lists approaches used in European legislation. It summarises the advantages and shortcomings of different elements typically used in available definitions, regarding their applicability in a regulatory context.

Indeed, as pointed out in the recent joint report from the JRC and the European Academies Science Advisory Council (EASAC) (2011), new initiatives in nano-specific training at the Master of Science and PhD levels are important to support inter-disciplinarity and to provide the next generation of nanosafety researchers with the skills to assess new generations of ENMs.

Conclusions

The International Risk Governance Council (IRGC) is an independent organization based in Switzerland that is focused on developing the concept and practice of risk governance. IRGC recently (2010) published a report on “The Emergence of Risks: Contributing Factors” in which it is postulated that emerging risks arise from a “fertile ground” that is cultivated by twelve contributing factors of which “scientific unknowns” is one factor. It is noted that “communication” has a particularly key role, as it can influence all the other factors. The twelve factors should not be understood as discrete units but as complex, interdependent factors. Moreover, the authors propose that the attribution of cause(s) to the emergence of risks should be informed by both reductionist and holistic inquiries. The latter “systems perspective” approach focuses on describing the system as a whole and not as the sum of its parts.
Three independent scientific committees provide the European Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The committees also draw the European Commission’s attention to the new or emerging problems which may pose an actual or potential threat. The three committees are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) and are made up of external experts. In addition, the European Commission relies on the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA). SCENIHR published a report in 2007 on the appropriateness of the current risk assessment methodology for new and existing substances for assessing the risks of nanomaterials. The aim was to assess the appropriateness of risk assessment methodologies described in the chemicals legislation for the risk assessment
of nanomaterials, and to provide suggestions for improvements. EFSA published a guidance document for the risk assessment of engineered nanomaterial applications in food and feed in 2011 (EFSA, 2011). The report is the first to give practical guidance for addressing potential risks arising from applications of nanotechnologies in the food and feed chain.

Nanotechnology is a multi-disciplinary field requiring the involvement of scientists in disciplines such as material science, physics, chemistry, biology, engineering, toxicology, clinical medicine, and social science. Similarly, nanosafety research also depends on close cooperation between material science, biology, and toxicology and risk assessment. This should be taken into account in the education of the next generation of nanosafety experts.
References


EFSA. Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Scientific Committee. EFSA Journal 2011;9(5):2140


To be able to flourish, the production of ENM and products incorporating them, as well as in a wide sense industry sectors utilizing nanotechnologies need a favorable environment to take the necessary risks required for new innovations. Hence, if one wants to promote the success of these key-enabling technologies (KET) as identified by the European Union communication on the new Programme for Research and Innovation - Horizon 2020, favorable environments for this research have to be created to reach such goals.

Safety has been identified by the EU Commission as a vital empowering issue for the success of ENM and nanotechnologies. Uncertainties related to the safety of these materials and technologies have created a major obstacle for industry and down-stream actors to invest into nanotechnology research and produce new exploitable innovations. Indeed, the EU Commission considers that concerns on safety related to ENM and associated technologies are a major bottleneck for the willingness of European companies in various industry sectors to invest into nanotechnologies, and hence Europe is lagging behind the US and Japan in this highly competitive area.

One needs to address which are the major success factors that enable flourishing nanotechnology industry. This document has identified several cross-cutting issues that are absolutely necessary for ensuring success.
Regulatory framework

Regulations

Chemical safety regulation in the EU is a structure based on two pillars. The first pillar is the legal framework for placing chemicals on the market, and the second is created from specific provisions for health, consumer, occupational safety and environmental protection.

i) According to the information given in the Communication “Regulatory Aspects of Nanomaterials” (EC, communication, 2006) all nanoparticles in chemical substances must meet the requirements of the REACH (Registration, Evaluation and Authorization of Chemicals) (Regulation (EC) No 1907/2006). Although there are no provisions in REACH referring explicitly to nanomaterials, they are included by the definition of a “substance”. The principal objective of the directive is to ensure a high level of relevant protection of human health and the environment.

ii) General requirements in relation to occupational safety and health of workers at workplaces are presented in the Council Directive 89/391/EC. The aim of this framework directive is to ensure a high level of protection of workers at work – including those exposed to nanomaterials - through the implementation of preventive measures to guard against exposure to risks, and through provision of information, consultation, balanced participation and training of workers and their representatives. The Council Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work describes the minimum requirements for the protection of workers from risks to their safety and health arising, or likely to arise, from the effects of chemical agents that are present at the workplace or as a result of any work activity involving chemical agents. Product legislation lays down the requirements regarding specific products, such as medicinal products, plant protection products, cosmetics, food and feed additives.

Consumer products that are not the subject of specific legislation have to meet the requirements of the General Product Safety Directive (Directive 2001/95/EC). Community regulation in these areas contains provisions in relation to health and safety of consumers, workers, patients and users. As nanomaterials contained in such products are a subject of REACH legislation, an assessment of their environmental impact must be conducted. All product legislation requires the performance of a risk assessment and the adoption of
risk management measures. Nanomaterials are not excluded from this obligation. The biocidal product regulation (Regulation (EC) 528/2012), which will be in force from September 2013, provides a framework of rules that apply to the marketing of biocidal products including nanomaterials substances and products (Directive 98/8/EC). The directive is intended to provide a high level of protection for humans, animals and the environment against results of use of biocidal substances. Environmental regulation relevant in the nanotechnology and nanomaterials context relates in particular to the Industrial Emission Directive (IED) dir 2010/75, the control of major accident hazards involving dangerous substances (Seveso II), the water framework directive and a number of waste directives. In principle, the IPPC Directive could be used to control for the environmental impacts of nanomaterials and nanomaterials issues at IPPC installations through the inclusion of such considerations into the Commission’s BAT Reference Document (BREFs) process should the need arise (Directive 2008/1/EC).

The EC Cosmetic Regulation (Regulation (EC) 1223/2009), that will be implemented as of July 1, 2013, requires cosmetic industries to provide specific data relevant for risk assessment purposes, and to notify the Commission of all the products containing nanomaterials six months before they enter the market. The labels of cosmetic products produced after July 1, 2013 also have to notify the consumers whether they contain nanomaterials.

The EU Regulation for Plastic materials and articles intended to come into contact with food (Regulation (EC) 10/2011) specifically states that “… authorisations which are based on the risk assessment of the conventional particle size of a substance do not cover engineered nanoparticles.” Hence requiring a novel risk assessment, and to date only three ENM have been authorized (titanium nitrate, silicon dioxide and carbon black). Finally, the last proposal for the Medical Devices Regulation includes some ENM specific requirements, considering all devices incorporating or consisting of nanomaterial “in class III unless the nanomaterial is encapsulated or bound in such a manner that it cannot be released into the patient’s or user’s body when the device is used within its intended purpose.”
Substances depending on their hazardous properties

For groundwater, EU Member States have to establish quality standards for pollutants representing a risk and in this respect, nanomaterials may also be included (Directive 2006/118/EC). The Directive on waste lays down the general framework and imposes an obligation on EU Member States to ensure that waste treatment does not adversely affect health and the environment (Directive 2006/12/EC). Hazardous waste must display certain properties set out in an Annex to the Directive and feature on the European Waste List as hazardous. Wastes containing nanomaterials could be classified as hazardous, if the nanomaterial displays the relevant properties which render a waste to be hazardous. Specific legislation has been adapted to deal with particular waste streams or specific waste treatment processes, such as incineration (Directive 2001/80/EC) and landfill (Council Directive 1999/31/EC). Current EU waste legislation is concerned with the general requirements for the protection of health and the environment during waste management. It also includes requirements for the management of specific waste materials that may contain nanomaterials whilst not explicitly addressing the risks of nanomaterials.

While the EU Commission originally considered the legal framework for nanotechnologies to be suitable in principle, amendments for chemicals, cosmetics and foodstuffs have subsequently been enacted. The primary force behind these amendments has especially been the European Parliament, and further regulatory changes (e.g. workplace safety, biocidal products, medicinal products, medical devices and waste) can be expected. Specific projects to evaluate the scope and requirements of possible modifications of relevant EU HSE regulations have been launched including the EU Safety& Health at Work legislation.

With respect to the REACH requirements, the REACH implementation projects (RiP) provided a gap analysis for nanomaterials. It was concluded that the existing REACH framework and the guidance can be considered as being applicable for nanomaterials, although a set of recommendations was given as a guidance update (RiP, 2011). There is a need for reference materials, standardized methods for identification/determination of physico-chemical properties and hazard assessment, and specific end-points that should be considered.
Developments

Based on recently started research into the needs for possible modification of relevant EU HSE regulations, and adjustments of REACH guidelines, the European Commission, and European parliament, together with the EU Agencies, EU Member State regulators, and competent authorities, should take decisions and provide further guidelines for the implementation of the regulations for nanomaterials. Legally binding limit values accepted by the EU Commission cannot be expected in the near- or mid-term future. However, expert-based provisional limit values/ reference values for a pragmatic precautionary approach may be developed in the short- and midterm.

Standardization

Standardization related to the safety of ENM will promote the spread of good practices and rationalize the communication between the authorities and the industry, and other stakeholders. The new European Standardization Policy proposal communicated on June 1st, 2011 emphasizes the importance of increasing the number of standards and to accelerate the development of standards in a fast-changing global landscape. This is particularly true for nanotechnologies that impact on many industrial sectors and where safety and social acceptance are important elements. In the new European Standardization Policy proposal, standards in the field of nanotechnologies are considered as very important because they can facilitate the introduction of new products by bridging the gap between research and marketable products, and also because they will contribute to the public acceptance of these innovations.

The Commission has, in accordance with Directive 98/34/EC addressed a formal standardization mandate to the European Standardization Bodies CEN / CENELEC Mandate M/409 for the elaboration of a program of standards to take into account the specific properties of nanotechnology and nanomaterials. This expresses the desire of the European Commission that should be an acceleration of the process of standardization of nanotechnology in general. In 2010, CEN, CENELEC and ETSI accepted the Mandate M/461 requesting the delivery of the standards listed in Annex I (Characterization of and exposure from nanomaterials) and Annex II (Health, Safety, and Environment) of the mandate. CEN/TC 352 “nanotechnologies” has drafted a road map, with potential topics to be adapted by the various TCs within CEN.
The contributions of researchers, e.g. by direct participation in Guideline development groups or external review groups, is a key factor for the development of standards. However, standardization activities in research projects are in general of low priority. The main commonly observed barriers for conducting standardization activities within a project are: lack of awareness among researchers of what is going on in standardization, lack of incentives for researchers to invest time and efforts, lack of resources to invest time for standardization activities.

Developments to promote standardization

CEN-CENELEC has produced a position paper providing a set of concrete proposals to integrate standardization within the framework of the Program for Research and Innovation: Horizon - 2020. This proposal includes reference materials and improved standardization opportunities in the frame of the Horizon - 2020. An integrated approach to initiate standardization as early as possible in the R & D promoting the innovations and research process, referred to as STAIR (STAndarisation, Innovation and Research) has been developed. The FP7 NanoSTAIR project builds on a sustainable process and platform in the field of nanotechnologies to support the transfer of knowledge to documentary standards in the STAIR framework. In this undertaking, bottom-up approaches will be used to identify standardization opportunities in EU and National research projects, and create platforms to support the transfer of research gained knowledge into documentary standards (see the NanoSTAIR turbine, Figure 3.1).
Figure 3.1. The nanoSTAIR process can be seen as a turbine that accelerates the preparation of new work item proposals by identifying the potential candidates, by making explicit the needs from the main stakeholders and by pooling the resources and expertise to reach the necessary critical mass. The turbine can be split in 4 steps:

(WP1) Research in the field of nanotechnologies is very intensive and fragmented over disciplines and countries. Some networks and associations are active to co-ordinate research investment. The main objective: to identify the standardization opportunities from research results.

(WP2) Possible new standard work items had to be clustered according to content, needs and possibilities. The main objective: to bridge the gap between the research objectives and the standardization needs.

(WP3) The verification of the approach will be done with the preparation of standardization work item for demonstrating and verifying the feasibility.

(WP4) The outcomes produced will be assembled to construct tools and guidelines to promote and translate in a practical way European nano-research into documentary standards.
The nanoSTAIR process can be seen as a turbine that accelerates the preparation of new work item proposals by identifying the potential candidates, by making explicit the needs from the main stakeholders and by pooling the resources and expertise to reach the necessary critical mass.

Apparently, the execution of Mandate M/461 has been stimulated by the Commission to assign a budget for the development of Standards and underlying pre-normative research. Some of the TCs and TC Working Groups have been very active in proposing standards and concurrent pre-normative research, for example in the area of worker exposure. It is expected that the availability of resources will significantly benefit the engagement of researchers in the process of standardization.
Conclusions

There is a large number of regulations and recommendations within the European Union and in the EU Member States that in many ways regulate the development and use of engineered nanomaterials and nano-enabled products as well as the development of nano-based innovations. It is extremely important that these regulations create a logical framework that at not only promotes the potential of novel nanotechnology innovations but also encourages the development of new materials. At the same time, the regulatory framework can be used to assure that safety will be taken on board in a consistent way in the development of novel engineered nanomaterials and nanotechnology products and processes. In essence, the regulatory framework should be one of the main drivers promoting the safety of engineered nanomaterials and nanotechnologies encouraging different parties to seriously consider safety as an essential part of the development of engineered nanomaterials and their applications. Hence regulations carry a very high potential to promote nanosafety. In addition to regulations, standardization is highly important in promoting the success of engineered nanomaterials and technologies, and when incorporating safety as a vital issue in the standardization process, these activities may become important drivers within European Union, EU Member States and beyond.

Infrastructure

Promoting those infrastructures that support nanosafety research within the European Union is a prerequisite for the competitiveness of European nanotechnology research, innovations and industries. This will require institutional support for organizations with permanent financial funding from their own governments, i.e. to research institutes, universities or industrial research laboratories.
Definitions and concepts

According to the European Commission Capacities Programme, the term ‘research infrastructures’ (RI) refers to facilities, resources and related services used by the scientific community to conduct state-of-the-art research in their respective fields. Examples include singular large-scale research installations, collections, special habitats, libraries, databases, biological archives, clean rooms, integrated arrays of small research installations, high speed communication networks, data infrastructure, synchrotrons and accelerators, networks of computing facilities, as well as infrastructural competence centres which provide a service for the wider research community based on an assembly of techniques and know-how. RIs are therefore at the centre of the knowledge triangle of research, education and innovation; producing knowledge through research, diffusing it through education, and applying it through innovation.

In short, research infrastructure means building, required research facilities and equipment, management structures of such infrastructures, and competences which are required for successful implementation of – in this case – of nanosafety research.

Nanosafety research is vital to enable commercially valuable innovations of nanotechnologies

Despite significant research and development (R&D) investment over the last 10 years, several critical road-blocks preventing the rapid implementation of commercially valuable innovations in a safe and responsible manner have been encountered. This fact is acknowledged by all stakeholders, and progress has not taken place to the expected extent. The real and perceived unknown hazards and risks of nanomaterials, allied to concerns about the reliability of current testing approaches, have been highlighted at many levels; from the scientists working in this area, from the media, even from high-ranking of government officials. To complicate the situation further, significant variations in the reported biological and toxicity outcomes of nominally identical materials have caused concern for the scientists and this has been reflected in, reports in media. This is a scenario for alarm and if not urgently addressed, it lead to a loss of confidence in science which in turn could destabilise nanotechnologies since it could lead to a loss of consumer confidence, a loss of public acceptance and to commercial disasters. Keeping this in mind, one should consider the various options about
how best to develop the infrastructure supporting nanosafety research so that relevant programmes and ideas can be effectively implemented. At the same time, it is important to understand that the contents of the nanosafety research are only indirectly associated with the requirements that need to be devised to create the desired infrastructure. This not only varies as a function of time and space and available competence requirements but is confronted by challenges posed by the emerging technologies.

One remarkable challenge to obtaining an optimal infrastructure is how to best utilize different required competences in order to resolve current and emerging research challenges, i.e. the governance and convergence of multiple scientific disciplines so that they can address this new challenge. The multi-disciplinarity of nanotoxicology means that it encompasses the fields of chemistry, biology, physics and engineering (Ostrowski et al., 2008).

The importance of strengthening of the nanosafety research infrastructure was emphasized by the European Commission and resulted in a project the goal of which is to improve an analytical Research Infrastructure for characterisation of nanomaterials for nanosafety assessment. As a result, the QualityNano research project was established.

A key element that emerges from the above analysis is that the development of even temporary or transient nanosafety research infrastructures may be valuable for supporting successful nanosafety research to further support effective safety assessment of nanomaterials / nano-products. This means that the assessment of nanomaterials safety should not be performed after the fact, or as a side project, but must instead it has to be an intrinsic step in the development of nanotechnology projects.

**Infrastructure and expertise for nanosafety assessment must be an intrinsic part of nanotechnology**

In order to build nanomaterial-based products which are safer by design, and to propagate best practice in nanosafety assessment it is vital to bridge the current gap between nanotechnology developments and nanosafety assessment. This is particularly important with regards to the research infrastructures involved in the production or in the processing of nanomaterials. One crucial stage is to remove, control and improve the stability of the quality of various types of engineered nanomaterials produced for commercial purposes, e.g. consumer products or downstream industrial applications. A European research infra-
structure for nanosafety assessment should be coupled with a state-of-the-art capability for nanomaterial synthesis, labelling and surface functionalization as well as equipped with facilities allowing animal and human exposure and ecotoxicity assessment. However, the most practical solution is that there should be a distributed set of facilities, linked by best practice. These facilities will share an underlying focus on quality and quality assurance, data quality and data sharing protocols, common databases to enable modelling and development of quantitative structure-property and/or structure-activity relationships (QSARs/QPARs), and they will work hand-in-glove with the regulatory authorities and relevant industry platforms such as the European Technology Platforms and industry organisations.

**Options for setting up European Union-wide long-range nanosafety infrastructure in the future**

When considering options that would most effectively enable setting up of infrastructures to conduct European Union-wide nanosafety research, one possibility would be to establish a single-site highly equipped facility with capacities to serve other EU nanosafety research facilities in strategic research areas. For example, these would include material characterization, and the performance of large-scale toxicity studies. The strategic capacities should also include the following services: bioinformatics, data management, utilization of systems biology approaches, and modelling of exposures and biological effects. Furthermore, another important support service for research and regulatory activities on engineered nanomaterials would be the capability to undertake risk assessment and the creation of databases and generating ontologies for nanomaterials. Finally, several other highly sophisticated techniques and methods, and substantial investments in research equipment and laboratory space would be required.

These types of investments could be situated in a stable organization with guaranteed fundamental resources into the foreseeable future, meaning that a long-range planning would be possible. For example, this kind of organization would be able to guarantee that there would be no changes of key personnel or short-term changes in research orientations due to unpredictable shifts in national research policies. Hence, there should be at least a governmental level, perhaps even an EU level, commitment to provide a long-term support for such a facility and to assure that this can be relatively long-term established centre of the required competences.
This option would require a long-term political commitment from either a single EU Member State, or the European Commission both in terms of financial support and ensuring the required human resources and competences. Due to the current financial situation and other circumstances, the establishment of a single-site European Union Nanosafety Centre is most likely not a viable option. In fact there is nothing at present which could be viewed as a gestational EU-level centre; its establishment would also require a high degree of consensus among involved EU Member States. To date, the required competences which would be needed in this kind of infrastructure capable of undertaking state-of-the-art nanosafety research are scattered around the European Union, and hence one need to seek an alternative solution to resolve this urgent issue and this needs to be addressed in the very near future.

One possibility could involve the mapping of available high-quality nanosafety research organizations, i.e. organizations with suitable space and laboratories, research equipment, human resources and competences, local funding and existing administrative support in research organizations within European Union Member States. One could also consider establishing a high-level group of decision makers representing different organizations and other stakeholders to assess possibilities for these kinds of endeavour with an associated aim to identify the relevant interested parties which possess the available resources with human competences and research equipment and laboratory spaces for different nanosafety research orientations.

In addition to discipline-specific analysis, also an analysis for discovery-driven and regulatory-supporting research facilities should be carried out. This undertaking, which is potentially realistic at the European Union level, could be executed in collaboration with the European Commission and the European Union Member States, and their existing nanosafety research organizations. All the parties should have a vested interest in supporting this activity. This endeavour could lead to the identification of a network of competence centres, i.e. research centres capable of meeting a series of relevant quality requirements.

The goal of the latter undertaking would be to establish a network of nanosafety research competence centres with different capabilities and objectives. These would consist not only of centres working on hypothesis and discovery/innovation approaches but also those with expertise focused on research serving regulations. This could also lead to fruitful cross-fertilization of both approaches. Even though there would be a number of legal and other obstacles along this path, it would allow the different parties to maintain their independence and hence would allow them to collaborate more freely which each other. Establish-
ing a European Union wide Virtual Competence Centre Network would allow a better integration of European competences directed to nanosafety research. This type of Virtual Centre based on a network of individual organizations would also mean that one could envisage the establishment of an EU Nanosafety Research Centre which would have a relatively light administrative structure e.g. with a rotating chairing of the administrative governance of the network. One important benefit of such approach would be that the associated organizations would have long-term basic governmental funding from their own EU Member State. The collaboration and networking structure could then be further complimented by temporary EU Commission funded projects providing support for network administration, mainstreaming of activities and an active networking visits of scientists between the partners.

Conclusions

A realistic option for EU serving would be the establishment of a network of competence centres, lead by the European Commission with the EU Member States’ support. Funding should come from the EU Commission to strengthen the networking and ensuring the execution of excellent research collaboration within this Virtual Nanosafety Research Centre with a light administration. The Centre would consist of a limited number of organizations that would quality as partners in this endeavor. One of the partner organizations would serve as the Coordinator of this activity to manage the day-to-day activities of the Virtual Centre and to streamline its activities of sharing of responsibilities and also the organizing of visits of scientists within this unique nanosafety research collaboration setting.
Innovation and value chain

In its Horizon 2020 Strategy (Europe 2020), the EU has identified priorities to promote smart, sustainable and inclusive growth throughout the EU and to promote the EU as the most competitive global knowledge-based society providing prosperity and social stability for its citizens. Innovation is at the core of this strategy.

Innovation and nanotechnology

Within the Strategy, the Commission has identified seven flagship initiatives to catalyse progress under each priority theme with innovation at the core of the activity. The Flagship Initiative “Innovation Union” aims to improve framework conditions and access to finance for research and innovation in order to ensure that innovative ideas can be turned into products and services that create growth and jobs. The Flagship “An industrial policy for the globalisation era” aims to improve the business environment, notably for SMEs, and to support the development of a strong and sustainable industrial base able to compete globally. At the heart of these activities is the concept that Europe will undergo an industrial transformation based on scientific and technological leadership and excellence.

In the Strategy, the EU has highlighted nanotechnology as one of the Key Enabling Technologies (KETs) to promote smart, sustainable and inclusive growth. As an “enabling technology”, nanotechnology should be applied early on and be a key element in the “value chain” being used to realise smaller, quicker, more powerful, or more “intelligent” intermediates and systems components for products with significantly improved or even completely new functions. (High Level Group on KET Report, 2010). There is huge potential for nanotechnology to provide employment and societal solutions.

Deployment of nanotechnology is a major driver for the trend to improve existing products by creating smaller components and better (in both functional and environmentally-friendly terms) performance materials. Engineered nano-
materials (ENM) and the technologies which utilise ENM represent one critical pathway to achieve these goals. EU FP7 project NANOfutures, the European Technology Platform (ETP) for Nanotechnology supports these aims by providing an integrating platform that brings together all relevant stakeholder groupings involved in nanotechnology. Specifically, NANOfutures aims to i) identify and optimise synergies between European and National Platforms, research programmes, JTI, ERA-NETs and other CSAs and research projects related to nanotechnology, in order to reduce the fragmentation of the European nanotechnology and coordinate future strategies; ii) identify key strategic nanotechnology nodes addressing issues of cross-sectorial and nano-specific relevance for the innovation and rapid uptake of nanotechnologies in order to increase EU competitiveness; and iii) construct and disseminate an integrated Industrial and Research Roadmap for European Nanotechnology.

**EHS barriers to innovation**

Issues regarding the safety of ENM, in relation to their potential adverse effects to human health and the environment, have been well-documented in the scientific literature giving rise to increasing concerns for regulators, as well as consumers and the industries that use ENM. An extensive programme of high quality research is currently underway at an international level in an attempt to address and alleviate these concerns. Europe is playing a leading role in this activity through projects funded under the ‘Nanosciences, Nanotechnologies, Materials and new Production Technologies (NMP)’ theme of the Framework Programme (FP 7), and its predecessor FP6, including the projects funded as part of the NanoSafety Cluster. However, these research activities have not yet yielded outcomes of sufficient clarity to address the many uncertainties surrounding EHS issues. In fact, the often contradictory nature of the research outcomes has been identified as a major contributor to the uncertainty.

The current debate, including the lack of regulatory clarity and, in particular, the uncertainty surrounding the potential risks of ENM have had a negative effect on the development, uptake and exploitation of ENM in the European domain and have been identified as a major barrier to innovation based on these technologies (EASAC-JRC, 2011). This has limited the extent to which these materials have been exploited through the value chain. The result of this has been a failure to fully exploit the potential benefits associated with ENM throughout innovation chain in Europe. Hence, major potential eco-
nomic benefits resulting from materials, processes and products based on ENM have been lost or at least significantly delayed. It is therefore of the utmost importance to overcome these barriers in order to open up these value chains and realise their full economic potential. This goal can only be achieved through the development of a sound science-based foundation from which to build a reliable and affordable safety framework.

**EHS solutions to innovation barriers**

The current programme of research being carried out in Europe as part of the NanoSafety Cluster activity is probably the largest coherent programme of research in any part of the world. The research activity underway already in part addresses many aspects of the innovation (value) chain. Projects have been concerned with among others:

1. Understanding mechanisms of toxicity (including ecotoxicity)
2. Development of dose-response relationships
3. Assessment of distribution, fate and behaviour of nanomaterials
4. Measurement and modelling of exposure
5. Development and evaluation of risk assessment methodologies
6. Assessment of life cycle issues

Activities have included strategic review and definition of state-of-the-art, networking, infrastructure development, assessment and adaptation of existing methodologies, fundamental research, application of evolving state of the art and dissemination activities.

However, the EHS programme of NMP research is not currently well integrated into the innovation-led FP7 work. Although many of the NMP projects have industrial partners, and are working with commercial nanomaterials and processes, these projects are more fundamental in nature and are concerned with developing an underlying knowledge, models and tools for subsequent application in risk assessment and management. Whilst this activity is critical to underpin the knowledge base, which will provide confidence in future nanotechnology-based products and processes at some point, it is important that EHS research should be organized that is more directly linked to the development of new materials, processes or products. Some projects of this nature are in progress, including NANCORE, ELECTROGRAPH and NANOMASTER. In these projects, the innovation aspects are being supplemented by an EHS work
package which attempts to address risk questions relevant to the specific application. Although these projects provide a direct route into the innovation process, the EHS component is typically rather minor and they tend to provide project-specific, rather than generalizable, solutions.

Fundamental research is critical and needs to be continued. However, it seems certain that the current projects will not produce all of the answers to all of the questions which surround nanomaterials. However one should not be overly pessimistic; it is clear that much better integration with the innovation chain is essential. A full Innovation Chain approach would allow a more targeted type of EHS research to be carried out. One relevant concept is that of “Corridors for Innovation” initially described by Grobe (2011). This was originally envisaged as a stakeholder dialogue concept that would develop a list of materials with a “licence to innovate” from researchers, regulators, risk assessors and civil society organisations. This would include examples of nanomaterials using specific limited types of formulation/processing for certain types of accepted applications which are “proven to be safe” over the entire lifecycle. This is a powerful idea in itself; however stakeholder dialogue alone is not sufficient to achieve this goal. Real research will be required to develop and optimise the solution. In these ‘corridors for innovation’ the spaces to innovate will be those areas where EHS barriers have been identified, defined and addressed. The challenge is to construct these corridors.

One option for implementation would be to develop large-scale EHS projects with the purpose of identifying, mapping, solving and acting as a curator to produce the solutions of the EHS issues for specific innovation chains. The dimensions of these corridors would encompass the entire life cycle including development, manufacture, use, release to the environment and end of life. The type of work that would be necessary would include toxicology, exposure, risk assessment, product safety, environmental release, fate and behaviour. The model for these projects is similar to the innovation-led projects that are currently on-going but on a much larger scale such that the outcomes are generalizable to the whole chain rather than simply being applicable to a specific application. A key aspect of achieving this goal will demand a shift in perspective from identifying what the risks are, to clarifying how we can find solutions that support and facilitate innovation.

In a general sense, EHS issues could be mapped and applied to the generic innovation (value) chain as depicted in Figure 3.2, requiring the development of both data and standardised methods to support better integration and innovation at each stage of the value chain.
3 Prerequisites for nanosafety research

Figure 3.2. Application of general EHS issues to the generic innovation (value) chain.

This idea is clearly generic, and implementation requires understanding and identifying the specific market-driven value chains within Europe, defining the major corridors of innovation within these corridors to which EHS issues could be mapped and subsequently addressed through targeted safety research. This approach should allow better alignment and integration of safety research into the innovation process and future technological developments. Through its road mapping activities, the NANO futures project is already in the process of identifying these specific value chains and mapping safety and sustainability issues, and some proposed actions, along the innovation pathway.

How does this link to the NSC Strategy

Many of the linkages within this approach to the NanoSafety Cluster strategy are already in place, as described in the rest of this document. There remains a need for research projects supported by the NanoSafety Cluster to be relevant, reproducible and of high quality, focussed on achieving scientific excellence. In order to address and overcome EHS barriers to innovation there is now the possibility of undertaking large-scale innovation projects that take custody of EHS issues in the whole innovation chain, identifying and mapping EHS issues to specific value chains, finding solutions to these issues and maintaining visibility and open access to the developing knowledge; these are the crucial topics that need to be addressed.
If we are to achieve this goal, then a change of attitude will be required, moving from a focus on clarifying what the risks are, towards a focus on identifying solutions that support innovation. Whilst fundamental research is critical and needs to continue, going forward better integration of targeted nano-EHS projects into the innovation chain is essential to support and promote responsible development and open up the ‘corridors of innovation’. These corridors need to be constructed around Europe’s prioritised and specific innovation (value) chains. The corridors need to be designed, built, reinforced with knowledge, rigorously defended and made open for business.

Whilst it is not the purpose of the NanoSafety Cluster activity to identify and define what these specific innovation chains will be, it should be their intention to work with those who do have custody of these value chains to ensure that a clear and coherent approach is taken. Only in this way will it be possible to guarantee that EHS issues are addressed and the barriers to innovation are overcome.

Conclusions

Appropriate EHS solutions, aligned with specific innovation chains and technological developments, have the potential to exert enormous impacts on the nanotechnology industry, regulators and society; they can provide positive and widespread benefits to the European Union and its citizens. Such solutions will provide a basis by which the toxic potential of all types of ENM can be understood and defined and provide industry, consumers and the regulatory community with reduced uncertainty and bring clarity to the current stormy debate swirling around the safety of ENM. The development and exploitation of appropriate solutions will support the innovation process by reducing the overall uncertainty concerning the safety of ENM but identifying in definitive terms which ENM in which applications are probably safe and which are not. Appropriate solutions will enable industry to make choices, early in the innovation path about selecting ENM on the basis of their characteristics and to prioritise the use of safe ENM in their products. Early identification of safe ENM and the use of these materials in the development of new products is an example of safety-by-design, i.e. by incorporating safety at the very start of the process. Hence, the likelihood of risks emerging at a later point will be effectively eliminated.
As a result, the various costs related to safety to the industry can be substantially reduced, enabling the manufacturing companies to focus their investment on safe materials.

EHS solutions mapped to the specific requirements of market-driven value chains will provide industry at all stages in the innovation chain with the confidence that the materials that they are using will not present future business risks (reputation, litigation) resulting from unforeseen safety problems with their materials and this will maximise and support the uptake of these materials in the development of new processes and products. The provision of reliable and relevant data will allow regulators to prioritise their activity to support the use of ENM proven to be safe, and hence to minimise the regulatory requirements for other ENM for which their safety has been demonstrated.

Appropriate solutions will help to relieve public concerns that their health and the environment will not be harmed, this will be achieved by clearly identifying those materials and applications for which there are effectively no safety issues. This will dramatically open up the possibility of widening the range of ENM and applications, free from concerns about potential safety issues.
Communication and dissemination

The current debate and the uncertainty surrounding the potential risks of ENMs may have had a negative effect on the development, uptake and exploitation of these materials throughout Europe (Owen et al., 2009) and this has led to a failure to fully exploit these materials in Europe and globally. This poses a special challenge to the communication and outreach related to ENM and nanotechnologies, and emphasizes the role of safety, trust and confidence as goals of these activities.

Targeted, neutral and reliable communication by the different stakeholders associated with nanosafety can markedly enhance the acceptability of safe and trustworthy ENM and associated technologies and to promote a new safety culture in nanotechnologies. Key-stakeholders include regulators, industry, various interests groups, representatives of media and the public at large. Public confidence in nanotechnology is paramount if these products are to achieve commercial success. Successful dialogue, dissemination of reliable information on nanosafety, and outreach to various stakeholder groups will all help assuring that health and environment aspects are being taken into account. This will dramatically open up the possibilities of widening the range of ENM and applications while still maintaining consumer confidence. Hence, these activities will support safe and confident exploitation of ENMs in a wide range of products and processes for the benefit of Europe and its citizens, and globally. One of the key stakeholders could also be the Virtual European Nanosafety Research Centre, whose Coordinator could act as a hub of wide-reaching and global efforts to distribute neutral and balanced and trustworthy information on ENM and nanotechnologies within the European setting and globally (see the section on Infrastructures).

Some major tasks shall be addressed by communication and dissemination activities.

Communication:

• Integration of all relevant key stakeholders (see below)
• Dialogue in focus groups to gain added value via detailed in depth discussion, this will involve representatives of all key stakeholder groups within European Union, North America, Asian countries and other global interest groups
• Active communication with press/media to facilitate science-based information transfer to the general public
• Setting up a framework which will bring together and coordinate the activities of national centres (established by national authorities in member states; see the section on Nanosafety Infrastructure and above) to exchange their results in scientific high-level groups on European level (bottom-up), as well as communicating back to the national level details about the best practices (top-down)

Dissemination:
• Standardized preparation and reporting of project outcomes, including all relevant information addressing different user-groups; e.g. regulators will need access to reviewed results, researchers will need raw-data-access, industry/investors will need up-scaling-information, etc.
• Shortening significantly the timespan from the scientific discovery to publication in peer-reviewed journals public by convincing the scientific journals to speed up the publication process
• Setting up scientific expert groups in defined focus groups to review the achieved outputs of projects, and to disseminate these results via a publication-tool which will be published by the European Commission (e.g. all data of projects have to be made available to the scientific community in templates, stringently structured to make results comparable and to find out to which level the information can be standardized so it can be the basis for further research)
• Development of a scientifically sound blueprint for nano-epidemiological studies e.g. including classification of exposure in epidemiological studies, a minimum set of biomarkers, general nano-assessment strategy, preparation of epidemiologic cohorts to ensure the health of exposed populations

Science-based communication and information-dissemination will be the key-elements in achieving sustainability of nanotechnological advances. However, the involvement of the regulatory community is vital because it needs to have confidence in the reliability of the outcomes emerging from the research endeavours. A sense of confidence between the regulators and other stakeholders would also provide flexibility and knowledge and form a sound foundation for decision-making for example refocusing research onto potential areas of concern. This kind of flexible dialogue and adequate communication may be one way to generate a step-change in the process of conducting risk assessment that will remove a massive testing burden from industry (which can represent a
major bottleneck for innovation in terms of both cost and time). This interaction will also enable regulators to simplify their procedures, removing another major bottleneck delaying the introduction of innovative products. The removal of these significant barriers to innovation will contribute to a quantum increase in the competitiveness of the European companies in world’s leading markets.

**Key stakeholders**

The key stakeholders in the nanosafety dialogue include the research and innovation communities, industry and consumer associations, standardization and regulatory bodies, various interest groups, NGOs and civil society, representatives of media and the general public.

**Research community**

One of the key aspects is the establishment of close and trusting working relationships throughout the different segments of the nanomaterial, nanosafety and nanotechnology scientific communities. One important collaborator in this context will be the EU NanoSafety Cluster (NSC), and the projects and organizations involved in the work of NanoSafety Cluster. This very document is an outcome of the joint collaboration by NSC partners, and this emphasizes the importance of assuring seamless collaboration within the NSC which has enabled its production. In the future it will be increasingly important to establish close working relationships with the scientific communities in the US and Canada, South America, Africa, Asia and Australia-Oceania. This can be based on the smooth cooperation already achieved within European community involving all of the relevant European countries. To reach these goals much more emphasis must be put on the dissemination and outreach of knowledge about nanosafety and establishing strong exchange programs for scientists. A head-start has already been made in creating a dialogue with the US research and regulatory communities by establishing the six Communities of Research (CoR) in different scientific disciplines (see Chapter 2 on Research Landscape).

Hence, there is still place for improving the collaboration within the NSC and the multinational European research projects through more vigorous dissemination of knowledge, information sharing between projects, exchange programs for junior and senior scientists, and overall more resource allocation
to dissemination and outreach programmes to enable genuine transparency in European collaboration of scientists, research groups and research organizations. A first step towards the strengthening of European science community will be the setting up of focus groups (e.g. nanosafety within NSC, nanomedicine within European Technology Platform on Nanomedicine (ETPN), nanotoxicology to be arranged using existing national structures), which will discuss the new information emerging from the projects by arranging periodical discussions. These can help to disseminate identified best practices throughout the European wide framework. Through this approach, the science community will become better connected, and this can speed up their developments compared to what can be achieved in international communities, endowing on Europe a leading role at the international level.

**Industry, innovation community and industry associations**

The ENM producers and the downstream industries need to be encouraged to adopt a novel safety culture e.g through the safety-by-design concept. This will require an active input from those companies which are in the forefront of the production of these materials and it will emphasize the special social responsibilities of these enterprises. In fact, these companies occupy a strategic position to have an impact on the whole range of other industrial sectors; by their own behaviour they would put positive pressure on all companies that produce ENM or are downstream users of these materials. The corporate image particularly of large companies is important for their success. A part of the strategic research activity can be the production of guidelines on how to encourage companies to utilize nanosafety by making them aware that this policy can polish their corporate image. The design elements of this corporate image still need to be further developed. Close cooperation and interaction with the research community will be mandatory for maintaining the pace of international market developments. The research community will benefit through undertaking cooperation with enterprises because of the relevance and business potential of the applications or marketable products, as well as via scientific merits for their track record. Equally, industry will be confident that it is bringing to the market safe nanotechnological products.
Furthermore, the reasons used by industry for its selection of materials i.e. how they are used and designed will also be crucial from the life-cycle perspective of ENM. This highlights the potentially important role of the industrial partners and the industry associations to which they belong. These associations include the Nanotechnology Industrial Association (NIA) and CEFIC.

This dialogue will be crucial for the successful dissemination of knowledge of innovations and ensuring the outreach of such innovations. This goal will require a close collaboration with the NANOfutures industrial platform and also an active interaction with the European Platform for Industrial Safety will be vital. These issues have been considered in detail when discussing how best to create the NANOfutures Roadmap. For example, NANOfutures will continually gather information on the needs of industry regarding the innovations emerging from the project. Thus, the contribution of NANOfutures will further amplify impact of the designated research priorities and its expected outcomes. Furthermore, collaboration with the other aforementioned key industrial associations, especially CEFIC and NIA, will provide this activity with the source data to be processed in the project.

Collaboration with industrial partners is a significant component of this proposal, the interaction with industry associations that have advisory roles and which enjoy close advisory interactions with the innovation-research community (NANOfutures, ETPIS) mean that the goals of the identified research priorities and this could be expected to extend their impact as they become incorporated into practice through regulation and standards.

**Standardization and Regulatory bodies**

Collaboration with CEN/CEENELEC and the ISO organization’s appropriate Technical Committees would be highly justified. However, to be involved in the standard setting either in the European (CEN/CEENELEC) or global standard setting (ISO) requires increased resources. These issues have been dealt with in an earlier chapter in more detail, but the issue is also relevant in this context.

In the European Union, the EU Commission plays a key role in regulatory activities within the European setting. Hence, from the dissemination and exploitation point of view a close collaboration with the EU Commission, especially DG Research, DG Enterprise, DG Environment, DG Employment, and DG SANCO will be very important. Other important interested parties within the EU setting are the European Chemicals Agency (ECHA), DG Joint
Research Centre, and the European Agency for Safety and Health at Work, as well as the European Food Safety Authority. All these key partners occupy key positions in promoting a positive change throughout the nanotechnology industrial sector a conceptual transformation making safety a crucial issue. This will provide regulators with the means to regulate the safe use of ENM and to promote safe industrial applications of ENM. The European Agency for Safety and Health at Work (EU-OSHA) working in support of the European Commission and the Parliament has the responsibility for collecting, digesting and disseminating information related to occupational safety and health within and beyond the EU. This body has also been active in the area of nanosafety. It can be viewed as a very effective organization for dissemination and publicizing the data emerging from this project. In combination and coordinated cooperation with the European framework, the effectiveness and impact of the activities conducted by both groups can be significantly increased.

It is important that there should be knowledge dissemination and an emphasis of the importance of the safety of ENM. For example, this can be achieved by devising the safety-by design paradigms and these are best created by working closely together with reliable global partners. Another key collaborator will be the Organization for Economic Cooperation and Development (OECD). It will be important to build a close working relationship between the various stakeholders in nanosafety with this organization. The collaboration is already well established but should be further improved by allocating more resources to highlighting collaborations with a global impact on nanosafety e.g. through the OECD Sponsorship Program on ENM. These contacts and outcomes of the NANoREG-project will be one way to ensure that this activity will have a maximal impact on the global industrial and regulatory communities and governments. There are other international organizations that play key roles in nanosafety dissemination activities e.g. the World Health Organization (WHO) and International Labour Organization (ILO).

**Interest groups and NGOs**

Business Europe representing European Union employers’ organizations and ETUC encompassing European Union labour unions are key parties voicing the views of European civil society. Both groups represent different, but important, sectors of the society, and hence are in a position to amplify the exploitation of the innovations of the project to their members. For this reason, both
parties need to be a part of the dissemination and outreach activities of the European strategic research endeavours on nanosafety. Non-governmental organizations and the interest groups at not only European but also the national levels are important and their viewpoints need to be heard during the development process of the nano-assessment strategy. By taking into account the views of these stakeholders at an early stage, it will make it easier to convince their members of the importance of accepting the key elements of the strategy i.e. from an occupational health and safety perspective as well as from general health and environmental points of view. Without cooperation with these groups, the development of a common strategy would be at risk of failing to be implemented.

**Media and PR**

It would be worth considering a central hub for dissemination of information and for outreach on nanosafety in addition to the work that is already being carried out by individual organizations. This would allow for coordinated and well-orchestrated efforts to achieve the goals proposed in the roadmap presented in chapter 6 of this document. A suitable candidate for the information and knowledge dissemination hub could be the Coordinating organization of the Virtual European Nanosafety Centre, as postulated in the above section on “Infrastructure”.

Media and PR work needs to be carefully targeted for different audiences so that the main messages associated with safety and benefits (science based knowledge) as well as the potential risks of ENM can be conveyed in a tailored fashion to the different target groups. The main groups include; 1) regulators at the EU and EU Member State level; 2) industrial sectors and companies; 3) industrial associations; 4) social partners at the EU and EU Member state level; 5) consumer and other interests organizations including e.g. Friends of the Earth, Greenpeace, etc.; and 6) the general public. In the future, governments and decision makers, international non-governmental organization, global enterprises and world-wide public audience through global communication networks, especially TV and various forms of social media will become increasingly important. These can be used not only to ensure that the message has a global impact but they can also be accessed to promote global networking of European stakeholders.
In this context, the means of conveying the targeted messages need to be carefully considered. In the case when the target group is small, such as major industrial companies, industrial association and regulators, one effective way to spread the message may be to organize workshops with carefully chosen speakers who have the ability to deliver the messages in a comprehensive fashion for that selected audience. These groups might also need to have dialogue and outreach events at the EU and at the EU Member State level. The small groups may include social partners, employee organizations and labour unions at both EU and EU Member State level. Tailored workshops or dialogue oriented events could be a useful way to reach out to different interests group both at the EU and the EU Member State level. A special message targeted to decision makers might be a highly useful approach for all of the above mentioned groups, especially those serving political and policy-level decisions makers and industry leaders.

When one considers industrial workplaces and the general public then the audience becomes huge; the interests of a vast audience will vary extensively, much more than in the smaller limited interest groups listed above. In this case, close contact with the mass media and carefully tailored delivery of the main messages would be highly advantageous, provided that the key media can be approached and convinced about the importance of the topic and the messages. The information intended for broadcast via the mass media should be delivered in all official EU languages and preferentially at a Member State level (top-down approach). Provided that some of the main drivers of the media attention, e.g. main TV news channels and leading newspapers can be interested, the delivery of the messages and dissemination of information by the mass media would be highly effective.

**General public**

Special attention should be paid to the way that scientific research findings are communicated to the general public. The general public’s confidence in science and research should be nurtured and one of the best ways is for the researchers to learn how to communicate their results effectively and attractively to the public at large.
Conclusions

The dissemination and outreach activities should strive to make all relevant knowledge emerging from the strategic research available to other qualified and relevant parties.

Networking activities should emphasize the translation of research and industrial knowledge to all relevant stakeholder groups. There is a pressing need to make society aware of the realities of nanotechnology and of the potential applications foreseen in the near future, including both the risks and benefits. Timely online sharing of data while allowing for confidential business information should be the goal. Global networking will be central in this respect. Openness and transparency will continue to be important parts of the networking, and this will only be achievable with a large degree of reciprocal trust. Efficient networking may require that new communication and networking paradigms are developed. The use of the Internet and participation in virtual forums and on-line cooperation will continuously influence the efficiency of networks, but the importance of physical meetings will still remain substantial, especially when developing and initialising new focus areas until common trust has been established. Centres of Excellence for nanotechnology networking should be created at the national level and supported by national authorities. Networking platforms should be financially supported or subsidised to enable new parties to initially access and enter the networking activities A European framework which coordinates the national centres should outline the synergetic potential of on-going research activities. It will be important to establish this central hub for dissemination of information and for outreach on nanosafety in order to facilitate coordinated communication and knowledge dissemination efforts.

It is noteworthy that EU funded project reporting takes place only through the European Commission. It would be advantageous to change this practice by installing a “database of research programs” which would act as a common reporting platform for all the project-outcomes to be collected using a standardized format and permitting comparison of all data. One location for such a database could be in the server of the proposed Coordinator of the European Virtual Nanosafety Centre (see section on “Infrastructure”).
It is crucial to amplify and to promote communication and dissemination of neutral and reliable information on ENM and nanotechnologies. Currently, the state-of-the-art in communication is (i) missing a critical review of research results at the national and international level, (ii) has no existing standardized communication rules or processes, (iii) has no coherent involvement of stakeholders in the scientific discussion and communication process, and (iv) has no coordinated way of overcoming these communication- and coordination-deficiencies. The coordination of all these activities needs to be strongly enhanced through better contacts between key-stakeholders in the nanosafety issues and by effectively using available mass media and other means of communication and dialogue to reach all the stakeholders and also the public at large, not just in Europe, but globally.

An important step would be the establishment of an effective dialogue between science and industry. This would overcome the present lack of knowledge-transfer. Industry should not feel that it is giving away precious secrets but should realize that it is gaining access to valuable resources. In this way, cooperation should be viewed a win-win situation for both science and industry.

It is crucial that the communication on nanosafety will encourage the translation of new safety-related discoveries to the core industrial concerns, to their business philosophy so that the promotion of safety-by-design concept can be easily explained and accepted by the different stakeholders, including the general public. This is an important area of research and innovations but its outcomes should not be obscured by poor communication. The incorporation of safety into ENM needs to be made visible through effective communication. This would allow an assurance of safety in conjunction with a guarantee of the commercial success of the nanotechnologies.
International Collaboration

International collaboration, both across the EU and globally, may provide a fruitful platform for having a larger impact and obtaining benefits in research as well as in aspects related to governance and safety issues of nanotechnology. In fact, large projects involving a set of demanding multidisciplinary, hypothesis driven research endeavours require international collaboration because in most cases the required expertise or resources may not be available in any one single country. Furthermore, international collaboration has its merits also because ground-breaking innovations often take place in the interface or cross-roads of different scientific disciplines and research environments.

The globalization of research is proceeding rapidly and this is having significant implications for the European nanosafety research landscape (see chapter 2 on “Research Landscape”). The forums of the production of new scientific knowledge are shifting from national to international arenas and comparisons of certain indicators across countries point to a positive relationship between measures of research collaboration and overall scientific impact (OECDa, 2011).

International partnerships create unique opportunities for enhancing scientific excellence, physical and intellectual research environments and innovative training of young scientists. Researchers gain greater access to information, ideas and facilities, which can facilitate the achievement of a critical mass and a more rapid advancement of knowledge and discoveries (EC, 2008). It is important to note that the driver for cooperation is not the cooperation itself. It is the goal of advancing better nanosafety research and knowledge sharing, bringing greater benefits for citizens and industries around the world in an era shaped by the need of addressing the major global challenges, many of which are closely linked to nanotechnology and thus to nanosafety research. International collaboration can take many forms ranging from mobility and physical cooperation to virtual cooperation, cross-border contract research, participation in international research organisations and, finally, to various levels of coordination activities and multi-stakeholder dialogue (Boekholt et al., 2008).
The European Commission’s Nanosciences and nanotechnologies Action plan for Europe 2005-2009 has called for attention to and action on issues of mutual benefit at a global level such as nomenclature, metrology, common approaches to risk assessment and the establishment of a dedicated database to share toxicological and ecotoxicological as well as epidemiological data (EC, 2005). Many international congresses, conferences and workshops have been held, addressing the environmental, health and safety aspects of nanotechnology. Progress has been achieved in many respects to identify the areas requiring joint efforts and the ways forward. However, EU or global level coordination is far from achieving goals of adopting international standards, nomenclature and databases, though important steps have been taken in that direction. Many obstacles or disincentives still exist, hampering collaboration across national borders and hindering the senior researchers or young talented investigators from working together. There is still a critical need to share knowledge in the health, safety and environmental aspects of nanotechnology and addressing the so-called nano-divide between the developed and developing countries.

**EU policy framework**

The research framework programme has been the key financial instrument to promote EU-led international cooperation in science and technology (Warriington et al., 2011). FP7 enables science driven international collaboration within the research projects since it is possible for research teams from basically all countries to participate in projects (provided they meet the requirements). In addition to the general openness to internationalization in the FP7 programme and support for international mobility and capacity building, specific third countries or regions can be included in calls for proposals for all themes under ‘Cooperation’ (EC, 2008). FP7 has also integrated the Specific International Cooperation Actions (SICAs) into the thematic programs.

The upcoming Framework Programme for Research and Innovation - Horizon 2020, will likely highlight the importance of international collaboration in research. As stated in the Proposal for the Council decision on Horizon 2020, international cooperation with partners in third countries is necessary to address effectively many specific objectives defined in Horizon 2020. Activities at the international level are important in enhancing the competitiveness of European industry by promoting the take-up and trade of novel technologies, for instance through the development of worldwide standards and interoperability.
guidelines, and by promoting the acceptance and deployment of European solutions outside Europe” (EC, 2011). However, FP7 or the upcoming framework programme, Horizon 2020, represents only a small proportion of all research in Europe as most research investment is by the Member States (public and private). The international collaboration opportunities in nanosafety should not be geared only towards the framework programme, but also multiple instruments and channels are needed in order to accommodate the research, policy and harmonization needs related to nano EHS issues.

In addition to the researcher-led cooperation opportunities provided in the framework programme, the European Union is signatory to Science and Technology (S&T) Agreements with a number of third countries, and these S&T Agreements provide another platform for collaboration. They enable institutional dialogue and link policy and science more closely together. Nanotechnology research is included in several EC S&T Agreements. The S&T collaboration between EC and the third countries is characterized by a top-down approach. Acknowledging that the EU cannot collaborate with every single country and in every field, the Strategic Forum for International S&T Cooperation (SFIC) has been mandated to drive forward the European partnerships for international S&T cooperation. To date, however, the SFIC roadmaps or activities have not directly emphasized the need for collaboration in the field of nanosafety research. The focus has been to a greater or lesser extent on the global grand challenges; however some of these are closely linked to nanotechnology applications and solutions.

**NanoSafety Cluster - from collaboration within EU towards joint collaborative EU-US research activities**

International cooperation has also become one of the key activities of the NanoSafety Cluster. In recent years, the European Commission has promoted dialogue with the US regulators and the US scientific nanosafety community based on the S&T Agreement between the EC and the US. Collaboration efforts between the European Commission and the US National Nanotechnology Initiative aim at bridging EU-US activity in the area of nanosafety research in close collaboration with researchers, regulators, and granting agencies.
Continuous discussions on global EHS research needs and challenges have led to a joint initiative to create EU-US Communities of Research (CoRs). The Communities of Research are voluntary activities of EU and US scientists and their goal is to promote collaboration in nanosafety research to be better able to face the global challenges related to the promotion of nanosafety research and its consequences. Source: EU NanoSafety Cluster

**Examples of multilateral cooperation and global initiatives**

A responsible and coordinated approach is needed to ensure that potential challenges related to nanotechnology are addressed. Multilateral cooperation links closely to the regulatory, standardization and risk-assessment activities. In other words, international collaboration in the development and introduction of standards for nanotechnologies will help early commercialisation, innovations, market development and potentially even contribute to better consumer acceptance. However, the multitude of actors (some of them described in Figure 3.3.) in international cooperation and global initiatives in nanotechnology poses a challenge also for the coordination of activities of EHS research. There needs to be a continual dialogue between scientists, researchers, policymakers and regulatory community to develop scientifically informed and evidence-based policies and ethically and socially sustainable solutions. An extensive international dialogue on future research needs and research activities is also important in this regard. Ideas to establish global consortia to solve global nanosafety challenges have been discussed and such ideas are perhaps increasingly on the international agenda.

Organisation for Economic Cooperation & Development (OECD), and in particular, the OECD *Working Party on Nanotechnology (WPN)* is in a central role in the promoting of development joint global initiatives on responsible development and applicability of nanotechnology between OECD Member States, OECD Observer States and other stakeholders. The WPN was established in March 2007 to advise upon emerging policy issues of science, technology and innovation also related to the safe of nanotechnology. It is a subsidiary group of the Committee for Scientific and Technological Policy (CSTP). The WPN works co-operatively with other OECD groups, including the Working Party on Manufactured Nanomaterials (WPMN, subsidiary to the
The Working Party on Manufactured Nanomaterials (WPMN) focuses on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. Its programme seeks to promote international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials. The WPMN is implementing its work through specific projects including development of databases, research strategies, test guidelines, and, more specifically, it has launched a Sponsorship Programme for testing a set of thirteen manufactured nanomaterials (OECD b, 2011). Each of them has a sponsor country and supporting countries on a voluntary basis. The first stage of this OECD Sponsorship Programme has ended and countries are seeking ways to initiate the second stage of this initiative. Moreover the WPMN has been developing guidance on how to apply chemical test guidelines for nanomaterial assessment and evaluating need for new test methods.

Other multilateral agencies include World Health Organization (WHO which also maintains a Network of Collaborating Centres in the nanosafety area), Food and Agriculture Organization of the United Nations (FAO), International Labour Organization (ILO), United Nations Industrial Development Organization (UNIDO), United Nations Environment Programme (UNEP), United Nations Institute for Training and Research (UNITAR) and United Nations Educational, Scientific and Cultural Organization (UNESCO). The UN agencies address nanosafety EHS aspects to varying degrees based on their own missions and activities and many of them are involved in the Strategic Approach to International Chemicals Management (SAICM). In the SAICM Process international scientific organizations have also played a role though governments have firmly assured that the SAICM process is preferentially an intergovernmental activity. When needed, the views of the global scientific community are sought.

The International Standards Organisation (ISO) is a non-governmental organization and the world’s largest developer and publisher of International Standards. International Standards Organisation Technical Committee 229 (ISO TC/229) works on standardization in the field of nano-
technologies. In the area of risk management, the *International Council on Nanotechnology (ICON)* is one of the key multi-stakeholder organizations for catalyzing global activities that lead to sound and responsible risk assessment, management, and communications and whose mission is to develop and communicate information regarding potential environmental and health risks of nanotechnology and hence fostering risk reduction and maximizing societal benefit. The *International Risk Governance Council (IRGC)* also works on risks related to nanotechnology even though this area is not its primary target. The IRGC is an independent organization working to facilitate understanding and management of global risks that impact on human health and safety, the environment, the economy and society at large. One of the informal global initiatives is the *International Dialogue on Responsible Research and Development of Nanotechnology* that has been facilitated through the UN Meridian Institute and in which individual countries have played an important role through informal intergovernmental collaboration. The European Commission has also been actively involved. This informal dialogue provides a space for facilitating international sharing and brings together various stakeholders to benchmark initiatives, identify differences and explore synergies, with the ultimate aim of contributing to a responsible and sustainable development of nanotechnology. Some International forums and initiatives on nanotechnology are presented in figure 3.3.
Figure 3.3. A diagram of international multilateral cooperation forums and initiatives on nanotechnology.

SAICM (Strategic Approach for International Chemicals Management) is a policy framework to foster the sound management of chemicals aiming at improving chemical safety globally.
Common Obstacles and Needs

Research can be identified also within other areas. Different legal systems and institutional settings can create difficulties and delays and represent a further administrative burden. Administrative and financial procedures regulating international cooperation contracts are often considered to be exceedingly complex and demanding (Warrington et al, 2011).

Furthermore, differences in ethical standards and IPR practices and legislation may be obstacles to collaboration. Lack of awareness and information about the cooperation opportunities and various funding instruments may many of the potential users of these cooperation opportunities are unaware of their existence. The multitude of funding instruments and constant project application cycles may also lead to “application fatigue” that could have negative impact on targeted calls on international collaboration. An open and transparent planning and implementation process of international calls and joint activities could enhance researchers’ interests and facilitate their activity and simplify call cycles.

International cooperation opportunities for nanosafety research should move beyond the opportunities offered in the context of the current and future framework programme. Complimentary aspects should include bottom-up collaboration opportunities in forming alliances and strengthening the international dimensions of the European Research Area in nanosafety research. This may require from the Members States a more proactive and coordinated approach. Nevertheless, there is a need for bottom-up, science-driven research and science-driven collaboration and this cannot be implemented from the top-down, but rather through a vigorous dialogue between the researchers in the field. Enhancing international collaboration in nanosafety research also requires recognition of differences in cultures and practices and reaching beyond the conventional, bureaucratic or short-sighted approaches.

Creating auspicious framework conditions would include:

- markedly improved information sharing between nanosafety research communities to avoid lack of knowledge of available opportunities for collaboration, and to promote integration of nanosafety research communities in different continents and regions
- better coordination and cooperation; optimizing the use of national, European and global resources and avoiding duplication of activities
• developing user-friendly frameworks and instruments in creating win-win situations (not just FP)
• allocating resources for international collaboration
• exploring and developing international co-funding mechanisms, establishing joint network(s), (virtual) institutes or joint programme(s) for nanosafety research
• supporting researcher mobility, optimizing various mobility instruments and continue the efforts for removing the barriers related to mobility (incl. competitive salaries and benefits for scientists)
• enhancing awareness raising activities and visibility about the collaboration opportunities and instruments (incl. horizontal and specific)
• supporting and meeting the urgent needs of nanosafety infrastructure
• creating mutual benefits and investing in the attractiveness of Europe as a top research destination
• political level initiatives and institutional frameworks or arrangements might open new doors in some cases (e.g. EC-EPA and EC-NIH collaboration)

International Dialogue in Nanosafety - towards a Global Research Area?

The international dialogue and collaboration activities have helped to understand the complexity of EHS aspects, further highlighting the need for joint international efforts in developing protocols and test methods to assess the health and safety impacts of nanomaterials and to provide proper characterization methods. Harmonizing ethical standards, IPR practices and development of common standardization practices would facilitate collaboration and conducting research, and thus ultimately, yield benefits to citizens and industries around the world.
Joint international efforts in the field of nanosafety are an important step towards the development of coordinated global collaboration and a move forward from the European Research Area (ERA) to a Global Research Area (GLOREA). One could say that steps in this direction have already been taken in the form of informal global initiatives and dialogues. Nevertheless, effective implementation of the ERA and its cornerstones should provide a basis for the development of the GLOREA. In this sense, it is clear that the European Commission and the Member States will continue to have a significant role in advancing multi-stakeholder collaboration and global efforts in addressing the responsible and sustainable use of nanotechnology and bridging the nano-divide. Despite the various actors in the international forums of nanosafety, there are no truly global institutions or instruments that would address the EHS issues in a comprehensive and coordinated manner. Perhaps the concept of GLOREA would thus reflect better the future mind set in planning and implementing policies and instruments that help bridging the gaps at the global level while finding a suitable balance between European and global interests.

An important future consideration of global research collaboration on nanosafety, or the establishment of GLOREA, is the role of developing countries and emerging economies in this activity. Emerging economies and emerging science countries such as China, Brazil, South Africa and Thailand are currently making huge investments into nanotechnology including nanosafety, and these endeavours should be integrated with the leading actors in the area of nanosafety, namely EU, US, Japan, Russia, Canada and Australia.

**Conclusions**

It is important to establish mechanisms and easy-to-apply-funding instruments (worth applying) for international, formal and informal, collaborative activities in nanosafety research. The opportunities for joint calls and co-funding should be explored. Multi-annual planning in cooperative activities and potential joint calls by the Commission and the member states and the third countries (allowing some degree of flexibility) would facilitate the planning and implementation of research activities of nanosafety research community and thus potentially generate larger participation and impact. Open and timely communication on collaboration opportunities is another of the key issues.
It is also important to create joint platforms or enlarging the current forums to include the emerging science countries in EHS research activities and to continue efforts to address the nano-divide (e.g. open-source databases). The GLOREA concept (starting with few third countries) may be one way to advance nanosafety through more coordinated and effective research efforts.

The importance of supporting international cooperation in terms of nanosafety research infrastructures continues to grow: an important area for progress involves mapping of the needs for large-scale international infrastructure investment, taking into account the European Strategy Forum on Research Infrastructures (ESFRI) processes in international collaboration and future RI roadmaps.

There is a clear need to intensity dialogue with the Strategic Forum for International Collaboration (SFIC) in nanosafety while engaging the researchers as part of the process. Continued efforts to remove the common barriers to international collaboration (harmonizing practices, IPR issues, legislation etc.) is another area that needs to be tackled.
References


3 Prerequisites for nanosafety research


3 Prerequisites for nanosafety research


OECDa (2011): Science, Technology and Industry Scoreboard 2011. OECD. http://www.oecd.org/document/10/0,3746,en_2649_33703_39493962_1_1_1_1,00.html


The NanoSafety Cluster (NSC) has arranged several meetings in which discussions have focused on the future key-challenges associated with the safe use of ENM and safety evaluation in the context of the nanotechnologies. At the same time, also the means by which this research could promote and enable major improvements in safety of ENM and lead to reliable, affordable, and faster than current evaluation of hazards and risk of ENM have been examined.

The key-issues identified have included a full understanding of the different characteristics of ENM, and the relationships of these characteristics with ensuring harmful effects of ENM on living organisms at molecular, cellular, organ and organism levels. Functionalization expands enormously the range of ENM available and this aspect has also been actively discussed. One of the key discussion topics has been the importance of characteristics of ENM for their penetration through biological barriers, and the interactions of ENM with various biological molecules upon entering a given organism. The effects on both human and environmental species have been thoroughly assessed. Naturally, before any effects can occur, exposure to ENM is necessary, and the understanding of the associations between ENM dose and effects in all organisms have been evaluated. Not surprisingly, the definition of dose metrics (characteristics) has triggered much attention and debate on which metrics of ENM, mass, number concentration or surface area should be used to define the dose of ENM e.g. in toxicity studies.

Substantial attention has also been paid to the potential exposures to ENM through the various steps of the production of ENM, the release of ENM at different stages of the life-cycle of ENM, and also from various surfaces, matrices, materials or products. The effects of wear and disposal i.e. through to the end of the material’s life have been considered as an important potential source of exposure in different environmental compartments. Exposure to ENM from nano-enabled products as well as the whole life-cycle assessment of ENM from cradle to grave has gathered considerable attention. Exposure routes including
aerosols, slurries and dispersions in processes, and importance of the release from products into the air or other environmental compartments from exposure point of view have been evaluated.

Recent developments advancing our understanding of the behavior of ENM in different matrices and environmental compartments, as well as in mammalian and environmental organisms have also been reviewed. A special emphasis was given to the foreseeable development of future types of ENM. Novel, emerging 2nd generation active nanomaterials, self-assembling 3rd generation nanomaterials and nanosystems, and the 4th generation systems of nanosystems and nanorobotics have been considered. It has been noted that most of the current safety research deals with the 1st generation ENM and the data on the emerging materials is quickly required. These considerations have been extended to all areas of the priority discussions, and hence implicitly included in all thematic areas identified during discussions.

Hazards have been extensively analyzed including translocation of ENM (absorption, distribution, metabolism, excretion), identification of the key-target organs of hazards, and the role of ENM-bio-interactions, molecular mechanisms, and protein and other coronas (phospholipid, sugars, nucleic acids) on the ENM characteristics and kinetic behavior. The special features of mammalian and environmental organisms have been carefully considered in this context. In addition, the development of various hazard/toxicity testing strategies that would allow quicker and more affordable hazard and risk assessment have been examined.

A high emphasis has been given to issues related to risk assessment and management of ENM. The high priority of novel risk assessment paradigms and the need to obtain human data have been considered in this context. Hence, the importance of worker surveys, exposure assessment field studies, epidemiological studies, and the establishment of exposure, company and worker registers in these contexts have been discussed.

Based on these considerations, conducted by all interested members of the NSC, the issues of nanosafety emerging in the near future and requiring rapid solutions have been identified. These four identified priority thematic areas are:

1) material identification and classification;
2) exposure and transformation;
3) hazard mechanisms including both human toxicology and ecotoxicology; and
4) risk prediction tools including databases and ontologies.
This text was prepared by an Editorial Group consisting of the chairs of the thematic areas, the coordinator of the NSC, and a few additional members volunteering to participate in this endeavour. These individuals were appointed to prepare a coherent document, emphasizing the identified priority research themes, preparing a roadmap for their implementation, and considering other elements to be included in this document.
4.1 Nanomaterial identification and classification

Most of the definitions of a nanomaterial concentrate solely on the size aspect (1-100nm), which misses the fact that nanomaterials are a very diverse group of materials with greatly varying properties. In order to enable prediction of impacts, a classification based on key parameters or biological interactions should be adopted.

State-of-the-art

Naming and classifying nanomaterials

There have been multiple efforts to define nanomaterials, including a focus on defining them for regulatory purposes which enables products containing nanomaterials to be identified and regulated, with limited success to date. Most of these definitions focus solely on size aspect of the nanoscale size (1-100nm) with some also including surface area and shape. There have been also been several suggestions for approaches to classify and prioritise nanomaterials for safety assessment, including the OECD Sponsorship programme approach based on commercial importance and volume of production.

The emphasis in the EU Commission’s definition on nanomaterials is on external dimensions, which may result in the exclusion from the definition of materials with an internal structure (e.g. porous materials with a relatively large internal surface area) or materials with a surface structure at the nanoscale. Further information is, therefore, necessary on the interpretation of information on nanomaterials in products and the impact of porosity (internal surface area) on the hazard of nanomaterials.

The proper detection and characterization of nanomaterials is a critical pre-requisite for the safety assessment of the materials under study.
The development of new and more robust methods and techniques for the detection and characterization of nanomaterials will also greatly improve the traceability and exposure assessment of nanomaterials present in consumer products. The results of recent research indicate that the size, size distribution and the surface properties of the particles are key parameters if one wishes to understand their behaviour.

The first and simplest characterization of NM should involve their particle size distribution (PSD); several techniques are available, but there is a clear gap in knowledge about the best approaches for a meaningful and cost-effective detection, size measurement and characterization of nanoparticles. An additional challenge exists for agglomerated ENM. Although the meaning of the term agglomerate is related to a measurable unit (i.e. external surface area), debate can still be expected on this issue. First, this assessment requires comparison of the surface area of the material with aggregates/agglomerates to the surface area without aggregates/agglomerates. The latter parameters can at present only be mathematically estimated from the size distribution of the primary particles, but this kind of mathematical estimation of the surface area is highly dependent on the quality of information on primary particle size. Furthermore, measurement of surface area is a common practice for powders, but no straightforward technique is (yet) available for particles dispersed in liquid. In addition, guidance on when the surface area of the aggregate/agglomerate can be considered to be the same or similar to that of the individual components is not available at the moment. In the Questions and Answers that accompany the Commission’s Recommendation of nanomaterials (EC(2011), it is clearly stated that aggregates and agglomerates are considered as being nanomaterials whenever the constituent particles are in the size range 1 – 100 nm. This is based on the fact that agglomerated or aggregated particles may exhibit the same properties as unbound particles. Moreover, there can be cases during the life-cycle of a nanomaterial where the particles are released from weakly bound agglomerates or under certain conditions even from more strongly bound aggregates.

A gap in terms of classifying nanomaterials is the fact that there are multiple different variants of each type of nanomaterial, all of which may differ in terms of their impacts. Thus, there is a critical need for an – International Union of Pure and Applied Chemistry (IUPAC)-type approach to naming and describing nanomaterials.
Approaches to group nanomaterials have been presented below:

1. **Classification by dimensionality / shape / morphology:**
   Shape-based classification is related to defining nanomaterials, and has been synopsized in the ISO terminology.

2. **Classification by composition / chemistry:**
   This approach groups nanomaterials based on their chemical properties.

3. **Classification by complexity / functionality:**
   The nanomaterials that are in routine use in products currently are likely to be displaced by nanomaterials designed to have multiple functionalities, so called 2nd-4th generation nano-materials.

4. **Classification by biointerface:**
   A proposal relates to the hypothesis that nanomaterials acquire a biological identity upon contact with biofluids and living entities. Systems biology approaches will help identify the key impacts and nanoparticle interaction networks.

Multiple reports have identified sets of physico-chemical parameters that should be reported for nanomaterials. However, not all properties are relevant for all nanomaterials, and many are not easily measured on a routine basis. An additional challenge is the fact that many of the physico-chemical properties of nanomaterials are context-dependent, and as such change depending on the surroundings in which the ENM are presented. Thus, we suggest the distinction between the *synthetic and biological identity* of nanomaterials. The *synthetic identity* describes the chemical, structural and compositional nature of the

![Figure 4.1. Nanoparticles and biomolecular interfaces: illustration of the concepts of synthetic and biological identity of nanomaterials.](image-url)
nanoparticles, including any surface coatings, ligands or labelling molecules; the *biological identity* describes the biomolecules that absorb to the nanoparticles under specific conditions and the impact of these on the dispersion properties, as shown schematically in Figure 4.1.

Clear guidance on parameters to report will improve data quality, enabling a cross-comparison of data and modelling and QSAR approaches. Over the next 5-10 years, the grouping of nanomaterials will become based on a deeper understanding of the characteristics of the nanoparticles and their interactions with their surroundings, and how these link to nanomaterials’ fate, behaviour and impacts on living systems.

In addition to the inherent variability in these materials, there is also an enormous level of evolution / transformation of nanomaterials properties, as they age, as they seek to reduce the energy associated with their surfaces, and as they interact with their surroundings. Assays to assess particle age, surface reactivity and evolution of surfaces should be developed and applied as standard as a part of the characterisation of nanomaterials.

Nanomaterials display a wide range of toxicities, with many being perfectly safe and other requiring some restrictions on their use to ensure that the benefits of application outweigh the risks for harm to human health or the environment. Thus, strategies that enable identification of low, medium and high toxicity nanoparticles are urgently needed. A set of so-called reference states should also be agreed to allow cross-comparability of studies and to allow the establishment of a baseline set of characterization data.

The OECD guidelines for Physical-Chemical Properties and Material Characterization were updated in 2010 and have now started to consider interactions with surroundings / characterization in situ as they now recommend the determination of size and size distribution dry and in relevant media (OECD, 2010). Given the capacity to interact with their surroundings, nanomaterials have both a synthetic and biological identity, which both may have an impact.

It is increasingly clear that the biological behavior and consequences of nanoparticles are largely dictated by how they interface with biology (Figure 4.1). These ideas are equally applicable to nanoparticles dispersed in environmental milieu, where decaying plant and animal matter interact with nanoparticles and thus affect their stability, dispersability and environmental fate and behavior. On this basis, we have introduced the concepts of the synthetic and biological identity of the nanomaterials; both of these properties need to be fully characterized in all studies in order to make the data meaningful, comparable and useful for predictive, read-across and grouping efforts.
### Table 4.1. Important ENM properties and common methods for characterization

<table>
<thead>
<tr>
<th>Physiochemical properties</th>
<th>Common characterization methods a, b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (distribution)</td>
<td>EM, AFM, DLS, NTA</td>
</tr>
<tr>
<td>Shape</td>
<td>EM, AFM, UV-vis (for plasmonic nanoparticles)</td>
</tr>
<tr>
<td>Agglomeration or aggregation state</td>
<td>EM, DLS, UV-vis (for plasmonic nanoparticles)</td>
</tr>
<tr>
<td>Crystal structure</td>
<td>XRD, ED</td>
</tr>
<tr>
<td>Surface chemistry/charge/area</td>
<td>AES, EELS, XPS, solid-state NMR, ζ-potential, BET</td>
</tr>
<tr>
<td>Stability over time/dissolution</td>
<td>DLS, UV-vis, ICP-AES, ICP-MS, colorimetric assays</td>
</tr>
<tr>
<td>Dosing metric</td>
<td>Variable</td>
</tr>
<tr>
<td>Uptake</td>
<td>ICP-AES, ICP-MS, TEM, fluorescence, flow cytometry, NAA</td>
</tr>
</tbody>
</table>

*aAbbreviations: EM, electron microscopy; AFM, atomic force microscopy; DLS, dynamic light scattering; NTA, nanoparticle-tracking analysis; UV-vis, UV-visible spectroscopy; XRD, X-ray diffraction; ED, electron diffraction; AES, Auger electron spectroscopy; EELS, electron energy loss spectroscopy; XPS, X-ray photoelectron spectroscopy; NMR, nuclear magnetic resonance; BET, nitrogen adsorption/desorption isotherm; ICP-AES, inductively coupled plasma atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; NAA, neutron activation analysis.

*bNot an exhaustive list of characterization approaches.
There are several techniques that can potentially be used to characterize these materials. Many of these methods have been listed in Table 4.1.

However, several problems need to be solved before reaching a more robust and systematic application of these techniques for the characterization of nanosized materials, especially in complex matrices such as real products and biological systems (see below). In particular, developing standard samples, samples preparation and analysis protocols, will be a mandatory step in making advances in this field.

**Detection and characterization of ENM in complex matrices**

It is becoming exceedingly clear that the behaviour, fate, and toxicity of ENM clearly depend on their surface properties. Thus, there is an urgent need for techniques able to characterize the surface properties (such as composition, chemistry, physical-chemical parameters) of NM both in pristine form, after the eventual engineering process and finally in complex matrices.

The current techniques available to measure NM size can be divided into three classes: 1) imaging-based, 2) light scattering-based, 3) separation methods.

1) Electron microscopy-based such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM). These techniques are the most frequently used ones to characterize NM; they are very accurate and can measure mixtures of NM of different sizes. However, they are complex and expensive, require some sample preparation and only a very small proportion of the total sample is actually analysed.

2) Laser light scattering-based such as dynamic light scattering (DLS), multi-angle light scattering (MALS), particle tracking analysis (PTA). DLS and MALS are rather simple to use, fast, relatively low cost, but can give misleading results when used to analyse samples containing non-spherical particles or particles of different sizes and in complex media. PTA calculates particle size on a particle-by-particle basis but suffers from a lower size detection value of around 30 nm.

3) Separation methods such as centrifugal particle sedimentation (CPS), analytical ultracentrifugation (AUC), and flow field flow (FFF) fractionation can be used. The major feature of CPS and AUC techniques is that they are effective in dealing with particle size mixtures. On the negative side, their accuracy may be compromised. FFF is a highly promising technique that can separate and measure complex mixtures containing NP of different sizes (down to 1 nm), it has an excellent dynamic range, and the various components can be recovered.
for further analysis. FFF suffers from a limited precision in measuring the absolute size of NP, but this limitation can be largely overcome by combining FFF with other sizing techniques such as light scattering.

Advanced / Emerging techniques
There are other techniques currently being developed that show great promise in being able to address some of the shortcomings of the more established techniques.

- Single particle inductively coupled plasma mass spectrometry (ICP-MS)
- Coulter counter and related pore-based sensing (e.g. iZON)

One possible solution towards an analytical platform able to measure the nanoparticle size distribution in complex matrices is the use of a multi-step approach where different building blocks are used in a sequential order. These building blocks would be: size separation, followed by size measurement, and particle quantification and eventual characterization.

There are also several approaches dealing with the chemical modifications induced by the presence of ENM in complex media such as blood plasma and their influence on potential oxidative stress induced by ENM. These cell-free high-throughput approaches are particularly promising and necessitate further investigation.

Beyond the State-of-the-art

Synthetic identity of nanomaterials
To date, most efforts towards classification of nanomaterials have been by composition / chemistry as shown in Table 4.2. Nanoforms of most conventional materials are currently considered as part of the bulk registration.
The limitation of this approach is that it does not include a detailed description of the surface coatings, stabilising agents, labelling entities, and other surface functionalization that constitute the nanoparticle. Additionally, it does not take account of the multiple different synthesis routes, each of which can lead to different physico-chemical properties (Napierska et al., 2010). This will become increasingly important for the description and categorisation of the more complex structural and functional nanoparticles, such as the so-called 2nd, 3rd, and 4th generation nanomaterials, which will possess complex geometries,
hybrid compositions and complex functionalities. In addition, the degradation of complex mixtures entailing nanomaterials can lead to products with the nanomaterials associated with other elements present in the mixture. The size of the product may not be nanoscale, and the product of degradation may not be toxic themselves, but they may become degraded into nanomaterials.

By 2020 there will be established norms for naming of nanomaterials, and the properties that can be correlated with uptake and impact will have been identified and validated as predictors of toxicity for new materials. Based on these properties, strategies for nanomaterials grouping will have been established, on the basis of application of quantitative property-activity relationships.

For certain nanomaterial types and media, the results obtained are different when data are assessed on a mass-dose basis or instead of via alternative metrics, such as surface area or particle count. There are studies where the effects were assessed using different metrics; the observed toxicity of the nanomaterials studied was deemed to be closely related to the surface area dose, and further, hazard categorisation of the different nanomaterials could be made in relation to surface area (Duffin et al., 2002; Duffin et al., 2007; Oberdörster et al., 2005; Stoeger et al., 2006).

Wittmaack (2007) however, indicated that particle number was a better dosemetric in nanomaterials hazard studies. Oberdörster et al. (2005), postulated that the surface area concept should be considered in the context of nanomaterial surface properties such as chemistry, charge, coating, crystallinity, porosity, and reactivity. Nevertheless, it is clear that there is no consensus on the suitable metrics to define the appropriate dose for ENM, not a surprising situation considering that so little is known on the association between ENM metrics and their biological effects.

Examples of environmental studies in this area are those of Van Hoecke et al. (2008; 2009). These authors assessed the effects of different nanomaterials (SiO₂ and CeO₂) on algae and Daphnia, respectively. In both studies, hazard results could be explained by differences in surface area. Consequently, they reported that toxicity was related to surface area and not exclusively to mass, even though there was nanoparticle agglomeration/aggregation. However, in most studies, often the surface area assessments have been carried out on dry particles, using BET, or even the data reported has originated from the manufacturer. Similarly it is unclear how particle numbers should and could be assessed given the tendency of particles to aggregate/agglomerate in liquid media. Therefore, although these results are important it is essential that technological developments will permit a more accurate measurement of surface area and particle
number in exposure media.

The strategy proposed to determine the correct dose metrics for nanoparticles will be to solve the analytical research gaps in order to obtain a clearer picture of the properties of the different nanoparticles and then, the most relevant dose metric could be suggested for groups of nanoparticles with well-defined characteristics.

**Biological identity of nanomaterials**

A hypothesis that emerged from FP6 and FP7-funded research projects relates to the hypothesis that nanomaterials acquire a biological identity upon contact with biofluids (e.g. river water, cell culture medium, blood or other bodily fluids) and living entities (e.g. cells, organisms, animals, humans, and that this corona of biomolecules determines their fate and behaviour. Significantly more research is needed to translate this into an implementable classification system, but the methods to achieve this are emerging, and systems biology approaches will help identify key impacts and nanoparticle interaction networks (interactomes). It has already been shown that the amount of uptake and the localisation of nanoparticles is dependent on the presence or absence of serum proteins, and that the nature and composition of a nanoparticle’s protein corona is dependent on the concentration of proteins to which the nanoparticles are exposed: thus the corona at low proteins concentrations (e.g. such as used for *in vitro* studies) can be dramatically different from that at higher protein concentrations (e.g. those present *in vivo*), suggesting that a reconsideration of how *in vitro* studies are designed in order to enable correlations with *in vivo* and human impacts (Monopoli et al., 2011).

It is important to note that interactions between nanoparticles and biomolecules, and the formation of the bionano-interface, has consequences for both the nanoparticle surface itself, and potentially also for the proteins and other biomolecules contained in the biomolecule corona. Note that a review paper on aspects of the bio-nanointerface and its role in determining nanoparticle fate and behaviour, based on the inputs to this strategic research agenda has been submitted to BioNanoMaterials, and contains additional details and discussion of this topic (Ahluwalila et al., 2013 submitted).

The composition of the biomolecule corona, and the subsequent stability, available dose and consequent biological interactions of nanoparticles, have been found to depend on the specific details of the biofluid in which the nano-
particles are dispersed, which may account for much of the contradictory reports present in the literature for nominally identical materials to date. Thus, the same (batch of) nanoparticles dispersed in different cell culture media (e.g. DMEM or RPMI) containing identical concentrations of Foetal Bovine Serum (FBS) have been shown to result in quite different coronas, both in terms of their thickness and dynamics (Maiorano et al., 2010). The authors of that study observed that DMEM elicits the formation of a large time-dependent protein corona, while RPMI shows different dynamics with reduced protein coating. These different coronas had implications for uptake and impact, with the protein-NP complexes formed in RPMI being more abundantly internalized in cells as compared to protein-NP complexes formed in DMEM, consequently exerting overall higher cytotoxic effects (Maiorano et al., 2010). These results suggest that cell culture medium composition and ionic strength can alter adsorption of proteins onto the nanoparticle surface, which can impact on the particle agglomeration and potentially alter the available dose of nanoparticles under the different exposure conditions. Thus, by not having the characterisation of the nanoparticles in the two different media, it is not possible to make any interpretation of the data on the basis of whether the different protein coronas result in different available doses, which could potentially explain the different observed impacts.

A study of the interaction of nanoparticles with surfactant protein A (SP-A), the predominant protein component of alveolar lining fluid (the first bio-fluid inhaled nanoparticles encounter) found different particle-protein interactions for each of eight different nanoparticles. Interestingly, three variants of the same material (cerium dioxide nanoparticles) revealed different adsorption patterns despite the materials being nominally identical and indistinguishable from one another in electron microscopy images (Schulze et al., 2010). This suggests that the protein corona composition (the bio-nano interface) could be a very sensitive tool to distinguish subtle material differences and for prediction of biological impacts, once firmer correlations between adsorbed biomolecules and signaling or other effects are confirmed.

Building on these findings, it is vital that nanomaterials be considered as biological entities, and studied as such – hence the introduction of the concept of a biological identity to complement the synthetic identity described above. A similar recommendation was made by an ERA SKEP – funded project “Nanomaterials in REACH” in its final report (SKEP), which recommended that ECHA consider NMs as biological entities and build on lessons from regulation of protein therapeutics and other biological substances.

Key steps in the short to medium term include understanding and eventu-
ally predicting which chemical, geometrical and physico-chemical parameters of nanomaterials lead them to preferentially adsorb which proteins, and connecting the absorbed proteins with observed impacts, such as uptake, localization and signalling. For example, the role of opsonins and dysopsonins is well understood in terms of phagocytotic recognition. However, much work is required to tease out the signalling pathways influenced by biomolecules contained at the bio-nano interface, whether these be functioning normally or experiencing altered functionality as a result of conformation changes induced by binding to the surface.

**Libraries of nanoparticles allowing grouping and nanoQSARs**: 

If we wish to advance the field, then there is an urgent need for sets of test nanomaterials, where the physico-chemical properties are systematically different. This would allow testing in a variety of end-points, which will enable application of QSARs. Coupled to this, is a requirement for certified reference nanomaterials for all end-points. Characterisation of the reference materials and the particle libraries under biological conditions is not a trivial issue, as the relationships between the nanoparticle properties and those of the surrounding media affecting aggregation/agglomeration state and the resulting changes of toxicity cannot be predicted based on current knowledge due to the plethora of factors involved in the interaction of nanomaterials with biological entities (Rabolli et al., 2010) that also include properties of the cell type being studied (Albanese et al., 2011).

Despite a growing amount of data devoted to nano issues, there are still no results of comprehensive, systematic studies within each of the nanomaterial classes. Even if a group of similar nanomaterials has been tested, the number of the considered group members is insufficient from a QSAR perspective. This relates to a key nanomaterials need; the creation of a series of nanoparticles which have their properties varied one at a time to allow systematic evaluation.

---

1nanoQS/PARs – quantitative structure or property-activity relationships for nanomaterials to allow grouping and categorization and eventual prediction of impacts from physico-chemical or biological properties (synthetic and/or biological identity.
**Labelled nanoparticles as tools for quantification of relevant dose-response relationships**

In order to correlate dose with response, labelled nanoparticles are considered as the best approach: these labels involve the use of fluorescently, isotopically or radio-labelled nanoparticles. However, from a regulatory viewpoint, the use of labelled variants is unproven as yet, and there are concerns that the labelling process itself may change the physico-chemical properties of the particles, resulting in different uptake and impact behaviours compared to the equivalent unlabelled particles.

Thus, there is an urgent need for labelled variants of selected classes of nanomaterials that are confirmed to possess similar properties, stabilities and interactions as their unlabelled counterparts. This may require core-shell models, as some research has revealed that labelling can dramatically affect the structure and stability of nanomaterials, limiting their usefulness for regulatory purposes unless such issues are addressed as a priority and the sameness confirmed.

**New approaches for hazard assessment enabling knowledge based grouping of ENMs**

It will be impossible to assess the risk of all ENM with all modifications and in all use scenarios using current case-by-case approaches. It is important to move away from purely descriptive toxicology of ENM to a predictive toxicology/nanosafety assessment, based on a thorough understanding of the dynamics of the biological behaviour of ENM derived from an understanding of their material characteristics. In order to identify the most relevant hazard-associated features as well as the most critical molecular signatures that predict the safety of the ENMs, state-of-the-art and beyond-the-state-of-the-art systems biology and bioinformatics approaches will need to be utilized. These novel approaches are being actively developed and some have been successfully applied in bioinformatics. By 2020, the ultimate goal will be to develop a computational tool, i.e. ENM SAFETY CLASSIFIER. This tool will predict ENM Safety based on the evaluation of minimal but sufficient amounts of information to provide a robust ENM safety classification. This novel prediction tool is clearly beyond-the-state-of-the-art but when available it will promote the utilization of safety-by-design principle, and also be capable of improving the speed of hazard identification and risk assessment. This tool will be tested and validated in close collaboration...
with industry; it possesses enormous potential to promote marketing innovations based on nanotechnologies.

By the end of the time horizon viewed in this document, it should be possible to assign certain biological effects to specific material properties and group ENM based on these material characteristics. Although, ENM have been shown to undergo interactions with cellular systems in vitro, this approach has only been partly successful to date. This will be a high research priority for the next years and it is anticipated that the correlation between properties of the materials and cellular functions will have been clarified by 2020.

As a complement, groupings may be based on similar biopersistence and biokinetic properties. Kinetics may also give a measure for grouping/summing nanomaterials. The same tissue distribution pattern may be one criterion for grouping of different nanomaterials. If a nanomaterial exhibits a different tissue distribution, this may result in different effects. Grouping may also be based on similar or common biological effects, including early effects and ENM-cell structure-interaction. Grouping based on early biological effects is tightly linked to the Adverse Outcome Pathway (AOP) concept of the OECD and the tox21 initiative in the USA (http://epa.gov/nctt/Tox21/). An illustration of the grouping of nanomaterials is presented in Figure 4.2, and further details can be found in the full manuscript developed from the inputs to the nanosafety cluster vision 2020 (Oomen et al., 2013).

Figure 4.2. Illustration of grouping of nanomaterials based on material properties or/and biological effects.
It will be necessary to combine exposure, biokinetics and hazard data for the purposes of both risk assessment and grouping. The AOP approach will provide guidance regarding the integration of material properties, exposure, biokinetics and hazard data. This is a priority for future research on the safety of ENMs.

**Rationale for the development of testing strategies**

A concern-driven guidance for toxicity testing of nanomaterials will be developed. This will enable focused research on nanomaterials that may be of particular concern based on expected exposure routes, material-properties as well as hazard and biokinetic data. This approach is in line with the 3R principle (Replacement, Reduction, Refinement), which focuses on the replacement of animal testing methods with alternatives that do not use animals, advocating that living animals are only to be used for crucial and focussed studies.

Based on the above information, integrated testing strategies will be developed for different types of ENM, starting by non-testing and existing toxicological data, proceeding with tests using acellular systems, and further proceeding through cellular systems to in vivo, long-term testing approaches when necessary. These strategies will be based on a thorough understanding of the matrix-dependent ENM biokinetics enabled by continuous sampling, analyses and characterization paradigm. The strategies will be based on validated methods with proven predictive power and they will be designed both to evaluate human health and environmental safety/risk assessment.

In 2020, guidance will be developed to determine how best nanomaterials can be grouped and how these groupings should be constructed. In addition to avoiding extensive hazard testing of nanomaterials, this will also provide insights when information on exposure and hazard for nanomaterials can be used for risk assessment purposes.

**Safe design of new ENM in a bottom-up approach**

The development and implementation of Safety by Design (SbyD) control strategies with its “primary” prevention value of risk management, represents one of the biggest challenge of nanotechnology that should guarantee its sustainable development.
Surface engineering has opened the doors to the development of a second, third, and fourth generation of ENM. Self-assembling bottom-up techniques have been widely developed at industrial scale, to create, manipulate and integrate nanophases into more complex nanomaterials with new or improved technological features.

Materials scientists have the chance to address such knowledge to the control of hazard specific properties by preserving nanoscale reactivity, towards the integration of safe-by-design approaches into the development stages of new nanomaterials and their applications.

The conceptual framework to identify key features that drive the design of safe nanomaterials (Roca et al., 2012) is reported in Figure 4.3. It includes a first level of data generation/gathering. The understanding of the mechanism that governs both the adverse effects of NMs on biological system and the emission/exposure potential in terms of fate from nano-aereosolization to bio-uptake is, in fact, fundamental to implement a rational approach for the safe design of nanomaterials. At a second level the observed evidences on nano-bio interaction mechanism should be supported by predicting models. Finally, at a third level the safer by design NMs should be implemented within real industrial processing lines, allowing cost-benefit analysis and the promotion of primary prevention based risk control measurements.

Figure 4.3. – Components of “Nano design” framework
Conclusions

A full understanding of the key descriptors for characterising ENM along with validated methods to identify and quantify ENM in complex matrices in vital in order to identify crucial parameters relevant for risk assessment. This is also important for the measurement of the relevant ENM properties that correlate exposure with biological impacts. This will require agreed reference states for nanomaterials characterization, libraries of reference materials, and a framework for understanding later generation nanomaterials.

The required research priorities to achieve this are to:

1. Develop systematic sets of ENMs with properties varied in a stepwise manner that will allow assessment of the significance of each property for toxicity.
2. Describe “reference” states and agreed media compositions to enable identification of significant biomarkers and enable a move towards a predictive toxicity assessment.
3. Understand the longer term fate of particles following their interaction with living systems.

Nanomaterial identification and classification approaches to determine the key descriptors that can be used to reveal correlations associated with impacts. The inter-relationship between the nanomaterials’ identification and classification is a cross-cutting topic and Figure 4.4 illustrates how it feeds into the other cross-cutting nanosafety research themes:
Figure 4.4. Inter-relationship between the sub-elements of Identification and classification and the other cross-cutting themes.
References


SKEP; Report from the SKEP project “Nanomaterials in REACH” http://www.skep-network.eu/Libraries/Network_documents/SKEP_Nanomaterials_in_REACH_Report.sflb.ashx


4 Common nanosafety research themes


4.2 Exposure, transformation and the life cycle

Exposure of humans and the environment is a result of many sequential or concurrent processes. These facts have emerged from research related to ENM production, ENM characterization, aging of products containing ENM, human and environmental induced release of ENM into the environment, transport, transformation, degradation and possibly accumulation of ENM in the environment or along the food chain.

Some of the above mentioned research areas have been formalized for traditional chemicals and products. There are some major harmonized or regulated activities e.g. OECD test guidelines for environmental fate studies, CEN standards such as dustiness tests for powdery materials and the regulation of chemicals Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH), just to name a few. The fundamental questions related to the existing frameworks have relevance to the now rapidly developing nanotechnologies, in particular those associated with the use of ENM, are:

- Is this existing framework appropriate to ensure the safe production, handling and use of ENM?
- Are the existing regulations and test guidelines applicable for testing nanomaterials, do they have to be adapted and/or do additions need to be made?

The current view is that the general existing regulatory frameworks are applicable but have to be adapted and extended for some ENM specific issues.
The clustered section below on exposure and transformation elaborates our current knowledge and the research areas which need to be addressed in order to derive accurate and reliable data for assessments. It has been emphasized that ENMs are the subject of some special properties, especially those related to the transformation of materials during their life-cycle or after their release into the environmental compartments which are known to alter their relevant substance characteristics e.g. size, shape, charge, state of agglomeration etc. Should that occur, this may modify substantially their hazard and exposure characteristics. Therefore, our knowledge with regard to transformation and current exposure models for conventional substance are not likely to be appropriate for the prediction of exposure to throughout the different stages of the life cycle.

**State-of-the-art**

Studies relating to ENM exposure have, to date, focused largely on occupational settings. Workplaces are better aware of the type(s) of ENM involved, and workplace exposures typically involve potentially higher acute and/or repeated exposures at relatively high concentrations. In comparison, far less research has been conducted on exposure to ENMs after their incorporation into consumer products. This remains a significant problem as exposure is largely dependent on patterns of usage and method of application. Research into the area of environmental release over the whole life cycle and exposure is confounded by its complexity due to the large number of contributing factors. This area remains in its infancy, despite the fact that there is an ever-increasing potential exposure.

In summary, it is clear that more information on the use of ENM, potential for ENM release and exposure in occupational, consumer and environmental contexts is urgently needed in order to derive a comprehensive overview of possible human and environmental exposures.
Figure 4.5. depicts the important steps involved in the exposure to nanomaterials. Two steps have to be considered for the determination of risk from exposure to nanomaterial. In the first step, release has to be identified in order that the exposure assessment can be conducted. In the second step, the transport and transformation of the nanomaterial has to be described to identify the type of potential exposure. After the type of exposure is identified, information about their possible health effects can be provided.
Figure 4.6. shows the life-cycle of a given nanomaterial and illustrates that any release will end up in some compartments of our environment. Consequently, this may then lead to an exposure of the environment, and humans through the environmental compartments.

![Figure 4.6. Nanomaterial life-cycle and release](image)

Environmental behaviour and fate determine the transport / mobility of the nanomaterials as well as their transformation. These processes influence also the likelihood of environmental exposure as well as possibly their hazard potential. An example is the environmental and biological degradability of nanomaterials. Unfortunately the number of studies examining the potential of degradability as a remediation technology is currently rather small.

**Life Cycle Assessment (LCA)** is the best developed and standardized methodology for assessing the environmental aspects and potential impacts throughout a product’s life from raw materials and energy extraction,
parts manufacture, assembly, distribution and sale, use and final disposition such as disposal, recycling, and energy recovery (i.e. cradle-to-grave). The environmental and resource impacts include climate change, stratospheric ozone depletion, toxicological stress on human health and ecosystem, the depletion of resources, water use and many others. The use of LCA also avoids the problem being simply shifted from one stage of the life cycle to another.

**Life cycle assessment (LCA)**

The ISO-framework for LCA (ISO 14040:2006) has been found to be fully applicable for nanomaterials and nanoproducts, even if data regarding the elementary flows and impacts might be uncertain and scarce (Klöpffer et al., 2007). So far only a limited number of research reports have addressed LCA of nanotechnology-based materials and products. In addition to the qualitative environmental assessments of the different manufacturing methods (Steinfeldt et al., 2007; Sengül et al., 2008), quantified material and energy flow data exist for only a very small number of manufacturing processes and/or for individual nanomaterials. It shows that studies have mainly focused on cradle-to-gate assessments. Cradle-to-gate is an assessment of a partial product life cycle; representing the stage from manufacture to its exit from the factory gate. The use phase and the after use phase (recycling, disposal) of the product are usually omitted (Meyer et al., 2009). For both of these phases, there is almost no data investigating their environmental impact.

**Long-term environmental effects**

The sensitivity of terrestrial systems to possible long term effects of ENMs to the environment has to be acknowledged in environmental studies, in conjunction with LCA. Water and air are mobile environmental media which will dilute ENMs concentrations and hence exposure levels will depend very much on emission rates, while there may well be long term accumulation of engineered nanomaterials in sediments and soils. This may lead to long term exposure to elevated nanomaterial concentrations but little is known about this possibility.
The LCA includes four stages:

1. Definition of the goal and scope of the investigation – description of the product system in terms of the system boundaries and a functional unit
2. Inventory analysis – collection, compilation and calculation of data
3. Impact assessment – data of life cycle inventory analysis is organized and summarized according to its environmental relevance
4. Interpretation – derivation of conclusions and issuing concrete recommendations

The relationship between these steps and the iterative nature of the LCA process is indicated in Figure 4.7.

Figure 4.7. Steps in the preparation of life cycle assessment [adapted from ISO 14040]
4 Common nanosafety research themes

**Release**

While ENM release is a prerequisite of downstream exposure, little has been done so far to approach this area in a systematic manner.

General processes and areas of possible release of nanomaterials include

1. Production
2. Handling and use
3. Aging
4. End of Life (EoL)

Possible release during production may occur through leaks into water and air in closed systems or open production processes. These have been studied in several European and national studies such as NANOSH, CarboSafe, and nanoGEM. “Handling and use” covers several process-related stages e.g. handling of powders, diffuse emission from production plants, mechanical treatment of nanomaterials, while “aging” encompasses all processes taking place in the environment such as selective degradation, wash-out, increased brittleness of the material.

End of Life activities refer to activities related to i) re-use or recycling, such as disassembling, and mechanical or thermal processes like crushing, melting, torch cutting, ii) waste treatment, e.g. incineration, and iii) disposal, e.g. landfill. In particular, during high energy processes, the release of nano objects may not be excluded.

Research and development activities aimed at understanding processes relating to release of ENM. This research and development activities are likely to increase in the near future since this will allow a) detailed studies of processes, b) standardised testing for certain possibly relevant release mechanisms, c) international harmonisation, d) derivation of quantitative information of possible release rates, and e) good characterisation of the physico-chemical characteristics of the released material. The importance of the latter has been nicely illustrated by Nowak and Bucheli (2007) in Figure 4.8.
4 Common nanosafety research themes

Exposure in workplaces

The workplace is generally the best characterised exposure scenario due to the relatively high exposure probability and the fact that there may be significant concentrations of nanomaterials being handled by workers and the relatively predictable nature of processes and activities. In fact, most of the studies conducted have been mainly exposure related or even more release related. No specific personal exposure measurements at various workplaces leading to a robust exposure assessment via inhalation or oral uptake have been conducted so far. Dermal exposure has virtually not been examined with the exception of Van Duuren-Stuurman et al. (2010) who utilized a shortened version of the observational DeRmal Exposure AssessMent (DREAM) to estimate the likelihood for exposure. This report highlighted the relevance of uptake via the intact skin (Grosera et al., 2009). With the exception of the intended use of nanomaterials in foodstuffs, possible oral uptake following inhalation exposure or from hand-to-mouth contact has not yet been examined.

All studies that have been conducted to date have only focused on short term exposure to ENM. As far as we are aware, there are no details of exposure monitoring or long term exposure assessments have been conducted. Another
topic to be addressed and in which major advances can be expected is related to the type of workplaces and work processes. The current workplace investigations have focussed on areas where nanomaterials are produced. Knowledge on use and processes with nanomaterials in the second or later stages can currently not be evaluated due to the lack of knowledge on their use. Labelling, which is also needed to identify possible exposure via consumer goods, is one way to address this safety research topic. Most exposure-related studies have revealed that mainly larger agglomerates and aggregates of Nano objects are released (e.g. Kuhlbusch et al., 2011) with only a few studies having clearly shown release and possible exposure to ENM that have at least one dimension below 100 nm in size.

Exposure via consumer products

The use of a wide range of consumer and food product types may result in different exposure scenarios, e.g. personal care products, cleaning, coating products etc. At present there are no relevant test scenarios of release and exposure to Nano objects. Current knowledge is limited to selected tests conducted for a few spray and cream formulations. In addition, we lack information on the NP content in consumer’s products and foods despite their widespread use. Information on their use and application is needed to permit a better evaluation of possible exposure sources and pathways. One of the main obstacles in studying consumer exposure is how to undertake a reliable measurement of particles in the different matrices of consumer goods and food products. Strategies to overcome this limitation will need to be developed. These may be based on testing of different release processes and realistic exposure scenarios for consumers using ENM specific measurement techniques and strategies.

Exposure via the environment

Environmental exposure to ENM represents potentially the most widespread mechanism for exposure, and therefore this is of relevance for the whole population as well as animals and plants. However, it is very difficult to study the interactions and distributions of nanomaterials in the wider environment, and when this problem is coupled with relatively low concentrations then scientists are faced with a major challenge. Environmental studies have so far been limited to
release related studies such as the report of Kaegi et al. (2008) which examined the TiO$_2$ wash-off from facades. Currently the main way of assessing possible environmental concentrations is using emission based approaches and models such as the technique utilized by Gottschalk et al. (2010). These workers extracted information on production rates, release fractions, assumed or based on measurement (e.g. sewage plant studies), and environmental transport and used this data to model environmental concentrations. These concentrations may be compared with environmental no effect levels for plants, animals and humans to assess a possible risk. Nonetheless, the application of the model is limited on a priori information and it would be advantageous to obtain better source data in the future. Currently no environmental monitoring technology is in place which could be used for monitoring of the environmental concentrations of persistent nanomaterials, concentrations of which are expected to increase in the future. This is certainly one area of future research, which although important, is currently not viewed as a straightforward task.

**Transport and Transformation**

It is accepted that nanomaterials may undergo various changes during subsequent processing activities and after their release, and changes may also occur during environmental transport. Transformation processes may take place in air as well as in liquid environmental media, soils and sediments. One can give examples of these kinds of changes e.g. loss of coatings, change in coating composition, development of a corona which depends on the particle surface properties, and dissolution in liquid media. Also other pathways of degradation exist which are not well identified or even used as a remediation technology.

**Beyond state-of-the-art**

**Release**

The major obstacle in studying ENM release, transformation and exposure is the identification of the particles themselves. Discrimination of particles by type (e.g. engineered vs. natural vs. particles produced during the manufacturing process itself) is of importance when assessing exposures, and in subsequent
analyses that interface with health studies. This problem increases as the ENM become ever more removed from the actual source both in time and space. For example, in the workplace environment, specific nano-objects are expected and release / exposure can be targeted using specific search criteria and protocols, and hence it is possible to limit resources to those parameters exactly fitting the appropriate purpose. Some strategies and techniques have been developed and tested in workplaces (reviewed in Kuhlbusch et al., 2011). However, severe limitations exist even for those used for research purposes and the existing techniques cannot be employed in routine workplace measurements. In the other extreme case, the environment, it becomes very difficult to develop an appropriate and feasible analytical method as nanomaterials may undergo modifications e.g. aging processes.

Another limitation is that currently there are very few measurement techniques that simulate aspiration efficiency and the deposition in the trachea-bronchial and alveolar regions resulting in mismatches between the concentrations measured, the concentrations inhaled, and the estimate of the deposited dose. In order to obtain health related exposure information, modelling techniques have to be applied to the data. This lack of health related exposure data, as well as some other factors, complicates the establishment of occupational exposure limits.

Measurement techniques and strategies are crucial in studying nanomaterial properties, behaviour, transport, exposure, uptake and fate. A few established techniques are currently available for these studies and have been summarised in the literature (Kuhlbusch et al. (2008), Tiede et al. (2008), Stone et al. (2010). In summary, the main techniques currently employed are either microscopic methods for information on particle morphology, state of aggregation and chemical composition, or methods discriminating particles according to size in relevant media. The latter methods sometimes allow subsequent separate analysis for chemical composition.

**Exposure in workplaces**

The next steps to pursue with regard to workplace exposure will be the development and testing of personal monitoring devices delivering reliable results that can be used in health studies and/or for risk management. Focus should be placed on personal real time instruments that simulate uptake, e.g. deposition in the different areas of the respiratory tract. The development of realistic exposure scenarios is needed to allow a comparative assessment of different tasks and processes. They
should be based on an extensive data set on workplace exposure, generated in a harmonized way as much as possible (Brouwer et al., 2012). The data included should be accompanied by auxiliary contextual information that is required to interpret the measurement results for risk assessment and mediation purposes. The exposure scenarios are also needed since they can be useful in to derive information about uptake for combined assessments of hazard and exposure potential.

**Exposure via consumer products**

During consumer usage nanomaterials are subjected to mechanical, thermal and environmental stress situations. Studies based on the characterisation of airborne particles release due to individual processes can roughly be classified by the investigated nanomaterial used for coating and according to the nanomaterials used in composites. Coatings could be considered to be a thin layer of composite material, as the engineered nanoparticles are intentionally embedded in a matrix material. However, in exposure studies, composites and coating cannot be compared and have to be analysed in different ways. The relatively long duration of the current aerosol measurement has restricted the intensity of abrasion. This means that with a higher abrasion intensity the coating could become worn off before the measurement finishes. Therefore only a limited simulation of exposure is possible. However, if one wishes to assess the real potential impact of nanomaterial on the environment and the human health, it will be necessary to characterise, with feasible techniques, the properties of the particles once they have been released into the environment.

**Transport and Transformation**

There are very rapid developments occurring in the fields of nanomaterial production and current technologies are not sufficiently well-developed to provide rapid assessments in a coherent manner. At present, some research groups are undertaking comprehensive research activities to develop some predictive models on how the material will interact with its surroundings, and how that may influence its subsequent transport, accumulation and reactivity; one must anticipate that there will be a huge increase in the knowledge base relating to ENM transport and transformation - including predictive modelling - occurring within the next 10 years.
Conclusions

The main goal to be achieved will be the development and implementation of integrated release to exposure models for nanomaterials in workplaces, consumer applications and the environment. One can anticipate that these will be based on the following ‘building blocks’:

Mechanistic understanding of processes determining the release of ENM
- studies on the behaviour of ENM when processed, when worked with, when being used taking into consideration possible nanomaterial release, aerolised or the presence of ENM in liquids
- comprehensive release and emission inventories covering production and all subsequent processing, usage steps and recycling

Understanding the transformation and transport of ENM
- studies on environmental mobility and transformations during transport and storage, including environmental persistence of the corresponding nanomaterial
- effects of ageing on nanoparticles, including changes in their shape, surface morphology and chemistry induced by environmental factors such as weathering, electromagnetic fields, mechanical stress and chemical reactions

Understanding workplace, consumer and environmental exposure
- efficient exposure measurement approaches also which can be applied in epidemiological studies
- harmonized inventories, which can be utilized in the construction of exposure models
- development of personal devices to estimate deposition in the respiratory tract
- evaluation of information relevant to describe exposure and inclusion of these factors into risk assessment and mediation strategies as well as into exposure modelling efforts
Another achievement by 2020 will be the integration of safe-by-design, closed production-to-product and green nanotechnology approaches into the development stages of new nanomaterials and their applications. The Social and economic benefits and/or problems should also be addressed in addition to the above mentioned aspects on exposure, transport, transformation and life cycle assessment if we hope to achieve sustainable nanotechnologies.

Exposure models for release-transport-fate of nanomaterials

- Identifying and process level understanding of key transformation processes in the environment and their testing.
- Advance and validate release process related models for exposure of workers, consumer, population and the environment, including diffusive emissions.
- Defining/prioritizing release processes; lab simulations and process level understanding.
- Integrating and advancing environmental transport models to include NM, their interaction with the environment as well as key transformation processes, combining all environmental compartments → link to effects e.g. by including NM uptake.
- Preparatory work for evaluated test methods for e.g. CEN, ISO, OECD etc.
- Set of well defined laboratory test procedures and evaluated models for use of product cycle and exposure assessment (for workplace, consumer and the environment).
References


4.3 Hazard mechanisms, biokinetics, and vulnerable populations

Hazard assessment of ENMs has made good progress during recent years, but knowledge is still lacking in many areas including modes of action and mechanisms leading to toxicity, identification of susceptible populations and vulnerable conditions, and aspects of biokinetics and its impact on toxicity.

Generating a comprehensive understanding of the biological behaviour of ENMs based on their material characteristics allows creating a knowledge-based ENM safety classification by the end of this decade. This can lead to the development of intelligent testing strategies for ENMs and would also provide solutions to other regulatory requirements related to ENMs, including affordable assessment of safety and classification and labelling of these materials. In addition, this research will also provide a firm foundation for resolving the safety classification challenges posed by the second, third, and fourth generation of ENMs.

The release of ENMs as in real-life situations and the dispersion of ENMs for toxicity assessment are of paramount importance for adequate hazard assessment. Since a multitude of different ENMs in various exposure scenarios is expected, all-embracing testing will not be possible. Hence, hazard assessment must be targeted to ENMs that constitute actual concerns in realistic exposure scenarios. Moreover, hazard assessment should be addressed by possibilities for grouping of ENMs and should also aid the grouping concept itself (see page 134).

Given the growing use of ENMs in a wide range of applications, it is predicted that increasing amounts of these materials will end up in the environment, leading to exposure of environmental species. It is clear, therefore, that ENMs should be considered as potential emerging contaminants. Knowledge
of the behavior of ENMs with respect to sorption, interaction with environmental organisms, accumulation as well as degradation is a prerequisite for the performance of scientifically sound hazard and risk assessments for ENMs.

**State-of-the-art**

Today, only a few EU funded nanosafety projects have, as their goal, the provision of a conceptual foundation, based on an in-depth understanding of the relationship between material characteristics and the mode of action (toxicity) of ENMs. This is done across species, and examining biological effects at the cellular, organ, and organism levels, to develop a general safety classification of ENMs. The emerging nanotechnologies are poised to deliver on the promise of promoting industrial growth and the economic wellbeing of EU citizens, but a solid framework for assessment of nanosafety is still lacking, despite much recent effort conducted in the EU and elsewhere. The deliberation of the new definition for a nanomaterial by the EU is one step forward, but the assessment of the safety of ENMs still proceeds as an *ad hoc* evaluation of new materials (EU 2011), an approach that will not be viable in the future, due to the lack of resources and the huge number of emerging ENMs.

Understanding of how nanomaterials interact with living systems is incomplete and, thus, we are not yet in a position to assess the relevant end-points for nanomaterial toxicity. At the same time, we are faced with an onslaught of new materials for which testing or screening of toxicity is required. To resolve this situation, methods for prediction of nanomaterial toxicity are needed.

There is uncertainty to what extend current methods to identify and assess the hazard of ENMs can be applied to ENM testing. When the modes of action of hazardous ENMs are poorly known, it remains unclear whether the assays reveal effects critical for the hazard. ENMs can be coated by all kinds of substances, either deliberately (coating) or unintentionally (surface layer), and very little is known about the impact of the resulting surface chemistry on both the toxicokinetics (absorption, biodistribution and clearance) and toxicodynamics (toxicity and recovery). The coating/surface layer has been show to influence both ENM biokinetics and toxicity at the level of a cell, a tissue or an organism.
Mechanisms of action leading to toxicity

Figure 4.9. shows a summary of possible mechanisms of toxic effects of ENMs. The toxic mechanisms of ENMs are still poorly known, and much of the information available on the adverse effects of ENMs is based on experimental *in vitro* and *in vivo* studies using relatively high doses. Most of the published toxicity data on ENMs derive from *in vitro* approaches, and it is presently still unclear, how well cell cultures could actually reflect the effects of ENMs *in vivo*.

Figure 4.9. Possible interactions of ENM with the cell and subcellular structures. (adapted from Colognato et al. 2012 with permission).

Inhalation exposure is considered the most relevant exposure route for potential human exposure to ENMs, and most toxicological *in vivo* studies published on ENMs have concerned the pulmonary inflammatory effects of ENMs (Colognato et al, 2012). Most of the oxidative burden related to particles is assumed to originate from increased recruitment of activated inflammatory cells, in particular granulocytes. Yet, the details of the inflammatory mechanisms
of ENMs are poorly known. In addition to inflammogenicity, relative biopersistence has been proposed to be another driving factor of the pulmonary toxicity of inhaled particles. Exposure to highly biopersistent ENMs of low toxicity may in fact result in a higher lung burden and associated inflammation than exposure to highly toxic ENM with low biopersistence (Pauluhn, 2013).

Poorly soluble, inert nanoparticles, such as nanosized TiO$_2$ and carbon black, are considered to represent granular biopersistent particles (GBPs) with no or little intrinsic toxicity. When inhaled, GBPs are thought to induce inflammation, oxidative stress, and, due to these phenomena, secondary genotoxic effects in the lungs (Hartwig, 2013). Nanosized GBPs may exert these effects at lower doses than microsized GBPs, although this question has not systematically been studied. High doses of GBPs appear to induce lung cancer in rats but not in other species; the carcinogenic effect has been associated with overloading due to particle deposition and retention on the respiratory epithelium, resulting in impairment of the clearance mechanism of the lung, inflammatory response, production of reactive oxygen and nitrogen species, epithelial cell injury and proliferation, and secondary genotoxic effects (ILSI Risk Science Institute Workshop Participants, 2000). Due to the suggested secondary mode of action of carcinogenic GBPs, cancer could be expected only in association with adequate, prolonged inflammation or oxidative stress. This would indicate the presence of a threshold exposure level below which carcinogenesis would not occur. However, very little is known about the carcinogenicity of nanosized GBPs and the actual mechanisms involved. In vitro studies actually suggest that nanosized GBPs and other ENMs could also have some primary genotoxicity, as seen by the induction of DNA damage and chromosomal alterations in cultured mammalian cells; in several studies, nanosized particles have shown higher genotoxic potency than larger particles (Schins et al., 2013). Although the observed increase in DNA damage in vitro is in many cases relatively low, it appears to be continuously produced (or persistent) in ENM-treated cells (Falck et al. 2009). The mechanisms behind these effects are not understood, but may involve, e.g., indirect effects of oxidative stress generated by ENMs in the cell or, in some cases, direct interaction of ENMs with DNA (Fig. 4.10.). As rather few comparative genotoxicity studies on EMNs are available in vivo, it is unclear, how well in vitro genotoxicity assays are able to reveal EMNs that are genotoxic in vivo.
Fibrous ENMs, such as carbon nanotubes (CNTs), may show, analogously to asbestos, fibre-like toxicity which depends on cumulative dose, the shape, dimensions, biopersistence, impurities, and other physicochemical characteristics of the material (Hartwig, 2013). Fibrous ENMs may have toxic effects on the lungs already shortly after single inhalation, possibly due to adsorptive depletion of essential homeostatic factors involved in surfactant homeostasis and subsequent dysfunction of the air-blood barrier, whereas volumetric particle overload may trigger retention-related responses affecting biopersistence and long-term sequelae (Pauluhn, 2013). Several studies have suggested that bolus exposure to ENMs by intratracheal instillation or pharyngeal aspiration may result in differential ENM lung distribution and different types of toxic effects than inhalation exposure. When injected intraperitoneally or intrascrotally, CNTs (especially long and rigid) have been shown to induce mesothelioma in mice and rats. The exact mechanisms behind these findings are not known. A number of in vitro studies have shown genotoxic effects with various types of CNTs, again suggesting some primary genotoxicity in cells treated with these materials. Figure 4.11. shows aggregates of carbon nanotubes in mouse lung.
Partly soluble metal-based nanoparticles, such as copper and zinc oxides, may show, in addition to particle effects, specific toxicity due to the release of metal ions. The nature of the toxic effects depends on the type of the ions and may involve metal-specific cellular interactions (such as genotoxic effects) in addition to inflammation and oxidative stress, at relatively low doses. Partly soluble nanoparticles may be more toxic than similar particles of larger size, possibly due to their higher intracellular bioavailability, even though this has not been confirmed experimentally (Hartwig, 2013).

**Biokinetics**

At present, there is very little integration of kinetic and toxicological effects testing for ENMs as for instance OECD Technical Guidelines for health effects testing substances do not require biokinetics. Biokinetics is not only important to increase our understanding on how (well) ENMs are distributed across the body, but the information is also used for interspecies extrapolations as well as for the design of follow-up longer term exposure studies and *in vitro* studies. Lack of integration leaves many questions open such as whether the data obtained during kinetics testing do apply to the results as obtained in the effects testing studies that is pivotal for reliable risk assessment. Most information available is for the inhalation exposure which indicates a complex deposition of ENMs based on their size distribution. For the oral route, the effect of the various physico-chemical conditions met during passage of the gastro-intestinal tract is largely unknown. For the dermal route, the release rates of cosmetic products and consumer products is largely unknown, as is the influence of sweat, temperature, sheer force, water as with showering and hand washing.

Absorption - Absorption is defined as the passive and/or active crossing of outer membranes. Currently, scarce information is available on rates of absorption of ENMs upon exposure via the three most relevant routes (oral, dermal and inhalation). Differences in rates of absorption are expected between routes and between ENMs and between an ENM and its non-nano counterpart. The information is still very limited due to the scarce availability of routine analytical equipment for particle analysis in these systems.

Distribution – Intravenous studies have indicated that ENMs distribute rapidly from blood to tissues, mainly those that contain phagocytising cells. The apparent very rapid blood/plasma half-life is in sharp contrast to the apparent long whole-body half-life. Distribution profiles may differ between ENPs with
different characteristics and different distribution profiles of ENPs may hamper combining hazard and exposure information.

Metabolism / Breakdown - Metabolism as known from classical soluble chemicals is probably quite irrelevant for ENMs as such. ENMs are regarded as too large to fit into the active site of biotransformation enzymes. Possibly, some oxidation may occur at the outer surface of ENMs. This may be related to Fenton chemistry of metals. These processes are generally not studied in biotransformation tests. What is more relevant for ENMs is twofold: first there is (time-dependent) dissolution into ions and secondly there is formation of any kind of surface layer such as corona formation.

Excretion - Excretion via the usual routes (biliary, urinary, via mammal glands, via saliva) is generally unknown. The rare studies available, however, suggest that excretion is very slow, which may be the reason for the sparse information available. The longer the half-life, the longer study is needed for a reliable assessment of the rate of excretion. This makes these studies relatively expensive, nevertheless highly relevant to assess terminal half-life; the larger the higher the risk for bioaccumulation.

Accumulation - Persistence is a main driver for bioaccumulation. It is believed that the structure and dynamics of protein corona is important to the rate of ENM uptake and transport into cells, and final subcellular localization. In this context, certain proteomics methods to identify the nature, composition and dynamics of the biomolecules associated to ENMs have been developed. Without suitable information on the potential for ENMs to bioaccumulate, it is infeasible to carry out higher-tier risk assessment or derive Environmental Quality Standards.

Presently, the hazard/risk assessment of ENMs can only be addressed on a case-by-case basis. However, given the large number of existing and emerging nano formulations, this will be a very time-consuming and resource-intensive task. In this context, Integrated Testing Strategies (ITS) have become particularly relevant since they are intended to speed up the risk assessment process and reduce testing costs and animal use. Good understanding of ENM biokinetics and translocation is crucial in the development of reliable ITS systems.

Susceptible populations and vulnerable conditions

The great majority of the studies exploring potential pathogenic effects of ENMs have been conducted in settings mimicking effects in healthy individu-
als, but only part of the world’s population can be categorized into this group. Many individuals have impaired health conditions that are likely to make them more susceptible to develop health problems from particulate exposure. The most relevant groups of potentially vulnerable conditions are: individuals with respiratory or cardiovascular disorders; individuals with chronic inflammation of the gut, or more permeable gut epithelium as a result of chronic diseases like diabetes or obesity. On the other hand pregnant women, children and elderly people can be considered as, individuals in a susceptible physiologic state. There are at least two reasons for identifying susceptible high risk populations and individuals with vulnerable conditions in the safety evaluation process of ENMs. The first one is that, even in the case of absence of adverse effects in normal healthy individuals, susceptible populations and individuals with vulnerable conditions may nevertheless develop a disease state after exposure; the second one is that they may require special protective measures in the case of the presence of adverse effects in the general population.

**Vulnerable conditions** - Currently, there is no direct evidence of the presence of groups highly vulnerable to ENM exposure. Their presence is suggested by epidemiological data concerning the ultrafine component of air pollution, but some experimental data regarding ENMs are now becoming available. Exposure to ultrafine particles present in air pollution exacerbate pre-existing asthma and COPD, increases inflammation and airway acidification. Experimental animal data show that intrapulmonary exposure to ENMs can aggravate pulmonary inflammation and airway hyper-reactivity. The rationale for the link between ENM exposure and asthma or COPD exacerbation can be made given that acute inflammatory events (e.g. infectious diseases) are known triggers of both asthma and COPD exacerbation and ENMs are known to be able to induce acute pulmonary inflammation. Epidemiological data link acute exposure to pollution-related UFP to sudden cardiac death, which is in part due to acute ischemic events. An increased mortality due to ischemic heart disease (IHD) has been reported in a cohort of workers exposed to metalworking fluids containing a substantial amount of incidental ENMs. Rapid thrombus formation has been reported in experimental animals after exposure to carbon nanotubes, and it has been also shown that inhalation of nano-TiO$_2$ decreases the ability of arterioles to respond normally to vasodilators. An important point to be taken into consideration is that both the surface chemistry of ENMs and the physiological state of the tissue are crucial for the behaviour of ENMs in the case of ischemic damage. Two events may lead to IHD in patients with CAD and both of them may be triggered by ENMs. Also the permeability of the gut epithelium is in-
increased in typical welfare diseases like obesitas and diabetes affecting increasingly larger segments of the population.

Susceptible populations - Epidemiological studies also indicate that exposure to environmental air pollutants is associated with adverse pregnancy outcomes, such as premature birth, reduced birth weight, small size for gestational age, and stillbirth. A high rate of severe malformations and abortions has been reported in mice after in utero exposure to low dose carbon nanotubes; severe malformations have also been reported after exposure to fullerene, but at very high doses. Milder effects on development were seen after exposure to silica and TiO$_2$ ENMs. TiO$_2$ ENMs have been shown to affect adversely the fertility and cerebral functions of offspring, whereas carbon black exposure was associated with genetic lesions in the liver of offspring. The reported experimental studies show that ENMs administered through several routes may reach both the placenta and the embryo, causing damage in both targets. In addition, they may be retained in the embryo, causing post-natal defects.

**Environmental fate**

It is expected that many ENMs will ultimately reach the wastewater. In this context silver nanoparticles are of interest, because they are used in many products of daily use, such as textiles, due to their antimicrobial activity. Furthermore, from silver nanowashing machines significant amounts of silver are released into the environment and finally reach the effluent of wastewater treatment plants (Farkas et al., 2011). Due to its physico-chemical properties, nanosilver is sorbed to sewage sludge. After degradation of the sewage sludge organic matter the metal will become bioavailable with a negative impact on the soil microflora. This result is not necessarily expected from the experiments on microbial degradation activity in wastewater treatment plants. These assumptions are supported by the findings of Oleszczuk et al. (2011) showing that the aging period of sewage sludge/CNT-mixtures affected root growth inhibition.

ENMs with a low sorption capacity may leave the wastewater treatment plant with the effluent resulting in a contamination of complex environmental compartments such as sediment and surface waters. Thus, sediment-dwelling organisms will be at particular risk to sediment-associated NMs which calls for a focus on research efforts to sediment/food exposure and subsequent accumulation and toxicity. However, thus far most environmental studies have focused on water exposures and a limited number of studies (e.g., from NanoReTox) have
included more complex matrices like soil, sediment and diet. Figure 4.12 shows
daphnids born in clean water and in water containing fullerenes.

Biodegradation is one of the possible environmental fate pathways for certain
types of ENMs. Specifically, any type of ENM containing organic carbon,
such as carbon nanotubes, may in principle be biodegraded. The possibility of
biodegrading ENMs also suggests the potential of induced biodegradation as a
means of remediating ENM-contaminated environments.

At present, there is some understanding of the behaviour of colloids in
the environment, but the details of how ENM behaviour relates to colloidal
behaviour, and how they interact with organisms are unclear. Consequently,
validated bioassays, hazard assessment tools, and especially predictive models,
remain to be developed and tested for ENMs. It is critical that underpinning
research be conducted that explores the fundamental principles that define the
consequences of the interactions of ENMs with biota. These interactions gov-
ern bioavailability, internal deposition, deleterious effects, and bioaccumula-
tion. In addition, long-term effects of ENMs on these compartments are largely
unknown.

Figure 4.12.
Two Daphnia magna aged 1-2 days. Upper
daphnid was born in fullerene exposure.
Fullerenes (black) fill the gut and have stuck
onto antennas, carapace, and thoracic legs of
the organism. Lower daphnid was born in the
culture medium. The gut is filled with green
algae, and antennas and carapace are clean.
(Photo by Kukka Pakarinen, UEF)

**Beyond the State of the Art**

**Mechanistic processes: nanomaterial biokinetics and translocation**

At the end of the time-horizon of this document, kinetics testing has been
integrated to a large extent in repeated dose toxicity assessment. Qualitative
(characteristics) and quantitative (concentration) measurements of ENMs along
the exposure pathway are in common use during repeated dose toxicity assessment. Standard integrated testing has been achieved by the widespread availability of nanomaterial-specific analytical equipment as well as expertise by toxicity assessment facilities. Total element analytical equipment such as MS is replaced or at least complemented by specific nanomaterial analytical equipment such as EM by 2020. Analytical capacity for qualitative and quantitative characterisation of corona constitution, agglomeration and aggregation status has increased dramatically.

Sampling and subsequent qualitative and quantitative analysis of ENMs is common practice while performing in vitro and in vivo human and environmental toxicity experiments. This has enabled standard establishment of kinetics in exposure studies as well as toxicity testing. Toxicity testing provides highly relevant data for various necessary extrapolations in risk assessment, such as; in vitro-in vivo, interspecies, high-to-low dose, and route-to-route extrapolations. Adequate information on biokinetics will allow various hitherto hampered extrapolations in risk assessment and change default factor extrapolation to data-informed extrapolations. By this development, important hypotheses have been generated as to the mechanisms of and the physicochemical properties that drive the (pre) biokinetics of groups of ENMs. This will facilitate ENM grouping and read-across within these groups by 2020 and waive kinetics and effects test for every single ENM in every possible matrix over time.

In 2020, the understanding of the mechanisms and significance of absorption, including de-agglomeration, of ENMs via different routes into and distribution and translocation throughout the body as well as cell type-specific uptake, breakdown and excretion will have markedly improved, and the significance of biological barriers will be better understood, allowing for a more reliable assessment of the possible health risks posed by ENMs to human health and environmental species. Kinetic data and kinetic modelling should become tools to evaluate whether an ENM behaves differently from another ENM or from the bulk material in order to assess if ENMs can be grouped for risk assessment purposes. Based on the mechanistic understanding, it may be predicted in which cases overload of various cell types will occur.

**Mechanisms of action leading to toxicity**

By 2020, the main toxic mechanisms of various types of ENMs are understood and sound criteria to classify ENMs for toxicity exist. Adequate comparative
data, on identical ENMs, from relevant genotoxicity and immunotoxicity assays in vitro and in vivo will have been gathered together with omics information from the same experiments, to judge which in vitro approaches best reflect in vivo toxicity and to see if other biomarkers or a systems biological approach could be applied to distinguish toxic ENMs. It will be clear which groups of ENPs can be classified solely on the basis of their physicochemical characteristics, which can exclusively be assessed by in vitro assays and which still require in vivo studies. Knowledge-based guidelines will exist on the correct way of conducting high-throughput in vitro toxicity assays with ENMs - not only for genotoxicity but also for. It will be possible to distinguish true genotoxic and immunotoxic effects that are predictive of an in vivo response. Validated in vitro methods will exist to identify carcinogenic ENMs with a non-genotoxic mode of action; for instance information on the ability of in vitro cell transformation assays to reveal carcinogenic ENMs will be available. Reliable and affordable techniques to determine intra-cellular ENM doses will have been validated and are routinely used in the in vitro tests.

The in vivo genotoxic and immunotoxic mechanisms of ENMs will be understood, e.g., with respect to the possible role of immunotoxicity in determining secondary genotoxicity. Guidelines will have been defined to conduct in vivo genotoxicity assays on target tissues of carcinogenesis, using appropriate exposure techniques and relevant endpoints. It will be known, if acute or sub-acute exposures should be used in short-term identification of carcinogenic ENMs with a genotoxic mode of action. A representative set of data will be available on the carcinogenicity of different types of ENMs, so that physicochemical characteristics, information on genotoxicity, and other relevant biomarkers can be used to classify ENMs for carcinogenicity. Alternative short-term in vivo test systems or biomarkers will have been developed to identify carcinogenic ENMs primarily acting via non-genotoxic mechanisms.

Special considerations in regards to susceptible populations and vulnerable conditions

Cardiovascular diseases - In individuals with existing cardiovascular diseases (such as atherosclerosis patients) in vitro experiments generally show an activation of key cells, such as platelets and leukocytes, and it has been shown that CNTs can promote activation of platelets collected from normal subjects. Currently, it is not known whether the same effect also concerns platelets and leukocytes
taken from patients with overt atherosclerosis. In 2020, this information will be available. Also, data on the possibility of an accelerated transition to atherosclerosis of disorders such as hypertension and diabetes that are known to increase the risk of this complication will be increased. Appropriate animal studies are advanced. The use of the Badimon chamber or similar *ex vivo* models to screen different ENMs for their potential to induce thrombosis in the presence of CAD is common.

**Allergic diseases and asthma** - Although considerable amount of information exits in the effects of ambient air particles on asthma symptoms and on allergic rhinitis only a little is known about the effects of ENM on these respiratory diseases. In 2020 knowledge about the induction of asthma and allergies by ENM is substantially increased. Effects of ENM exposure in asthmatic patients as well as in patients with skin allergies (e.g. contact allergies and atopic eczema) are investigated in sophisticated experimental *in vitro* and *in vivo* models. Some of the finding will be selectively validated also in human subjects. Moreover, ENM are classified based on their potential abilities to induce or exacerbate different types of diseases (including allergies) using advanced computational models and user-friendly web-based interfaces.

**Pregnancy**- Knowledge of ENMs’ ability to cross the placenta and reach the embryo and the possibility of late post-natal effects will be increased. As the permeability of human placenta changes during the course of pregnancy, the appropriate experiments on ENMs at different stages during pregnancy will be performed. The fact that embryo toxicity may develop even in the absence of placental crossing by ENMs, if they are able to damage the placenta itself, will be taken into consideration. The placenta represents the only way by which the embryo may obtain oxygen and nourishment and its substantial damage unavoidably will cause embryo injury. Currently available *in vitro* and *ex vivo* models will be examined for an adequate number of ENMs and validated in parallel in *in vivo* studies.

**Elderly and Babies**- In 2020, there are specific studies on possible augmented effects of ENMs in elderly and babies. In elderly subjects the probability of atherosclerosis, even in the clinically silent stage, is much higher than at younger ages; therefore the probability of an accelerated course of the disease may be higher in this group. Both in elderly and young the skin is thinner than in healthy adult individuals, and the studies showing minimal or no penetration of ENMs through the skin will be re-evaluated in these groups. In general, the immune response and defensive capacity is hampered in these two age groups, with immature immune system in young and with age-induced de-regulation of reactivity in the elderly.
Environmental effects testing

Standardized ecotoxicological tests are suitable for the comparison of chemicals with respect to their ecotoxicity and for a first risk assessment. For refined risk assessments tests with more realistic environmental conditions are required.

Implementation of standardized ecotoxicological tests for the effects of ENMs in the aquatic environment may be problematic as aggregation/agglomeration is concentrations dependent such that agglomeration increases with increasing concentration in the water/media. Consequently, a 3 dimensional exposure scenario will occur resulting in a non monotonic concentration-response relationship (i.e., decreased toxicity with increasing overall concentration). There exists some information in the literature suggesting that nanoparticle size affects accumulation of metal NPs even after being mixed into a complex compartment such as the sediment. This calls for further studies on the fate of ENMs in sediment, and subsequent consequences for accumulation and toxicity. In addition, accumulation appears size-dependent only in invertebrates with more complex digestive systems. Since there is evidence for delayed effects of ENMs, future work should consider long-term effects in the aquatic environment. The research on the terrestrial environment needs to focus on long-term effects in realistic environmental concentrations of ENMs. The present investigations on mainly single species have shown effects in a concentration range much above the environmentally realistic level (mg/kg level).

In general, there is still a need for investigation of individual species differing e.g., in feeding mode (filter-feeding vs sediment-ingestion) and digestive complexity, but effects on populations, communities and ecosystems should also have high priority. Long-term tests at realistic concentrations and microcosm- and mesocosm-based test systems may offer environmentally more realistic scenarios. This will enable the assessment of the interaction between different species and the relative sensitivities of organisms and also enhancing the basic for selection of species for further standardized testing.

ENMs will most often reach the terrestrial compartment through application of sewage sludge on agricultural soil or landfills or via deposition from the air. ENMs will also reach the aquatic environment via sewage treatment plants and end up in the sediment compartment. Passage through a sewage system as well as the fate up to soil deposition and after introduction to the aquatic environment and subsequently in the sediment, will most likely change the ENMs significantly. These changes have to be considered and accounted for in future research. The influence of the dosing or mixing of the ENMs in a test
system has to be assessed. This requires an investigation of medium preparation and of methods for spiking of test materials and effects of possible carriers.

Beyond the more traditional endpoint included in e.g. OECD standard tests, multiple endpoints have to be investigated. This includes assessment of different biomarkers, such as oxidative stress, but also uptake of ENMs in tissues and behaviour of organisms. Knowledge about the fate of ENMs in the soil is one of the key topics in the design of terrestrial effect studies and for the environmental risk assessment. There is a well-documented need for research on the transformation of ENMs in water, sediment and soil systems. This includes agglomeration/aggregation kinetics, sorption and abiotic/biotic changes of ENMs in the food, sediment, soils, water and pore water. Reliable methods for characterization and quantification of the ENM before and during the testing period are necessary prerequisites for future research.

Conclusions

Mechanistic knowledge should be included in technology development, to help the safe design of new ENMs in a bottom-up approach, and will feed directly into the development of a rational testing approach.

The key factors in developing knowledge and understanding the toxicity of ENMs are:

- identification of the main modes of actions of toxicity for ENMs
- understanding the transformation of ENMs during their life cycle and how this may influence their hazard potential
- identification of the key physicochemical determinants that modulate ENM interactions and toxicity in biological systems

By 2020, the following should have been achieved:

Hazard assessment enabling grouping of ENMs

1) Scientifically sound grouping criteria
2) Understanding the association between material characteristics and perturbations in cellular events
3) Guidelines for high-through-put toxicity screening
4) Utilizing systems biology approaches in the prediction of ENM safety

Biokinetics including translocation and clearance (dissolution and excretion)

1) Biokinetics are much more integrated into toxicity testing
2) Mechanistic knowledge resulting in groups of ENMs with similar kinetic modes of action is obtained
3) The mechanisms of the bioaccumulative properties of ENMs are investigated with highest priority by studying terminal whole body half-lives of various groups of ENMs

Susceptible populations and vulnerable conditions

1) Systematic research of ENM effects on susceptible populations (validated in vivo and ex vivo models for pregnancy, children and elderly people)
2) Systematic research of the effects of ENM on individuals with vulnerable conditions (validated in vivo and ex vivo models for diseases e.g. cardiovascular diseases, allergies, diabetes etc)

Environment

1) Fate of ENMs in complex media such as food, sediment, soil, water and porewater
2) Improved prediction of the (bio)degradation rate of organic nanomaterials help to describe the long term fate of ENMs in the environment.
3) Development of standardized test methods for water (hard, soft, brackish, marine), sediment and soil
4) Establish relation between physiological factors (feeding mode, digestive complexity) and ENM accumulation and effects.
5) Effects under more ecologically relevant conditions: effects on populations, communities, and ecosystems e.g., by using long-term effects, mesocosm-based test systems.

6) Further development of biodegradation research to assess the potential of plant enzymes as a means of remediating carbon nanotube contamination in the environment.

Key aspects of hazard assessment

- Development of concern-driven testing to include NM type, their interaction with the environment as well as key transformation processes, combining all environmental compartments ➔ link to exposure (fate and behaviour)
- Development of High Throughput Screening (HTS) approaches and Toxicogenomics and High Content Analysis (HCA) systems
- Link to regulatory testing (e.g., OECD work)
- Understanding uptake processes; experimental work with well characterised NMs and systems (Development of PBK models)
- Understanding of characteristics - fate - effects relationships ➔ link to grouping (sameness)
- Set of well defined laboratory test procedures and evaluated models for use of product cycle and hazard assessment (for human and the environment)
- Development and validation of QNAR approaches
- Approaches to evaluate possible effects in vulnerable systems

<table>
<thead>
<tr>
<th>2015</th>
<th>2020</th>
<th>2025</th>
</tr>
</thead>
</table>
References


As the scientific basis of risk assessment (RA) for ENMs suffers from substantial limitations, both communication and dialogue are urgently needed with respect to risk management (RM) driven desired or approved actions. Databases and epidemiological or health studies can be considered as enabling ‘tools’, supporting the processes of RA and RM.

Traditional risk assessment frameworks follows the four-step paradigm:

1) hazard identification
2) hazard assessment
3) exposure assessment
4) and risk assessment

Risk Assessment (RA), Risk Management (RM) and Risk Governance (RG) are closely related. RA can be considered as the scientific backbone of the process of risk governance, whereas RM represents its subjective political part. Clearly, the RG includes the communication and dialogue between stakeholders and the areas of knowledge generation (RA) and decisions on actions and implementation (RM) (Renn et al., 2005). As the scientific basis of RA for ENMs has substantial limitations, the communication and dialogue are urgently needed on RM driven desired or accepted actions. Informatics (data bases) and epidemiological or health studies can be considered as enabling ‘tools’, supporting the processes of RA and RM. For example database studies which enable predictive hazard modeling, e.g (Q)SAR, PBPK or exposure modeling.

Epidemiological studies can provide data supporting both hazard and risk assessment. Clusters of a given hazard in an epidemiological study can serve hazard identification or hypothesis formulation, and odds ratios or risk ratios found in epidemiological studies may serve the evaluation of the relevance of toxicological findings to human health. Furthermore, epidemiological studies may serve hazard and risk assessment by identifying unexpected potential biological adverse effects, or finding vulnerable (sub) populations.
With respect to RM, the outcome of epidemiological studies could be important to assign or evaluate various threshold limit values such as occupational exposure limits (OEL) values in occupational settings for workers, or acceptable daily intake (ADI) values for consumers. The association between RA, RM, RG and databases and epidemiological studies is illustrated in the modified International Risk Governance Council (IRGC) Risk Governance Framework (Figure 4.13).

**State-of-the-art**

Risk assessment (RA) for ENM with respect to the life cycle of these materials is challenging for several reasons. After their production, nanomaterials may be transformed, e.g. by agglomeration or de-agglomeration, or by loss, change or...
development of coatings, which may have impact on the uptake and biological effect after uptake. The information about the level of exposure to ENM is fragmented, most of the studies are rather explorative and the results cannot realistically be used for an estimation of the exposed dose. Predictive exposure models are mass-based and this parameter might be less appropriate in cases where one wishes to evaluate the risks associated with a nanomaterial. To estimate the impact of a health risk, one needs to be aware of the number of people that could be exposed. This number relates very much to the penetration of nanomaterial-based products in the value chain and for the time being, accurate information is lacking (Pronk et al., 2011). Clearly, most of the parameters of the risk assessment process involve uncertainties and this results in high uncertainties when one tries to estimate the overall outcome of this process. The same issues also apply to environmental risks, where during and after release, transformation reactions are even more important in changing the properties of the pristine nanomaterials (Gottschalk and Nowack, 2011). The conventional RA framework may fail to estimate the risks from ENMs due to overwhelming methodological limitations and epistemic uncertainties. The present paucity of quantitative nano-EHS data will lead to ambiguous, qualitative risk estimations, based on expert judgments that may fail to be reflected in appropriate and timely regulatory decisions. Currently, quantitative risk assessment has been translated into operational requirements by using risk ratios, i.e. observed dose of exposure divided by a reference dose or exposure limit.

To date, for nanomaterials, only a few reference doses or health-based limit values have been proposed (Schulte et al., 2010); (NIOSH, 2010; 2013). Alternative risk analysis tools and frameworks as well as modifications to existing risk assessment approaches have been proposed for nanomaterials.

To provide the risk assessment process with adequate information with respect to the hazard of ENM, there are several issues that are of prime importance:

- Development of ‘grouping’ strategies and nano-QSARs to identify high concern ENMs and predict relevant endpoints of toxicity and ecotoxicity
- Development of standard test methods and validation of relevant in vitro models
- Characterization of the hazard in terms of quantitative dose-response relationships, relevant for establishing threshold limit values
• Characterization of the hazard in terms of quantitative time-response relationships, relevant for the development of a reaction
• Globally harmonized epidemiological studies to validate biomarkers and to prevent/assess health effects in a longer perspective, and relevant field study approaches to assess potential effects of ENM at the population level of different environmental organisms
• Extrapolation from \textit{in vitro} to \textit{in vivo} (animals and man) and \textit{vice versa}

Currently, there is a paucity of human exposure data; most workplace studies have focused on the emission of nanoparticles and consequently the potential for exposure rather than on quantitative assessment of concentration at the breathing zone (Figure 4.14.). There have been very few estimates for exposure further down in the life cycle, and since in most cases the ENM are matrix embedded, the likelihood of an exposure is considered to be low. Some tiered approach exposure assessment strategies have been developed. However, scientifically sound decision criteria to evaluate the release with respect to potential for exposure are lacking. For risk assessment or epidemiological studies, it is not clear which expression of exposure is relevant, in fact it is not even clear what metric would be most appropriate for evaluating the amount of ENM present.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image.png}
\caption{Exposure assessment during liquid flame spray process (Photo by Joonas Koivisto, FIOH)}
\end{figure}
With regard to the fate and behavior in environmental compartments, it has been anticipated that nanomaterials in aquatic systems will tend to agglomerate/aggregate and potentially sink, resulting in general low availability, even though clearly conflicting results have been recently reported e.g. suggesting that sediments are not likely to be the ultimate sink of ENM in aquatic environments (Pakarinen et al., 2011;2013) (Figure 4.15.). Some nanomaterials may exert detrimental effects on the activated sludge from waste water treatment systems (WWTS). It is still unclear what are the environmental levels of exposure; some estimates have focused mostly on modeling with little regard for actual real-life exposures.

If one wishes to conduct a realistic risk assessment of nanoparticles, it is extremely important to identify the physico-chemical properties that predict different toxicological outcomes. ENM are complex groups of materials with diverse physicochemical properties, which not only can affect their biological activities but also their underlying mechanisms of action. For the same reason, it is as important to identify the behavior of ENM interacting with biological systems. They behave totally different than larger (micron) particles.

A suite of risk prioritization tools have been developed to indicate the need for exposure control and risk management. However, only a few of the exposure assessment components of these tools have been calibrated, and none of them have been extensively tested or validated. The hazard component of these tools is mainly based on the interpretation of toxicological data of the parent material. Uncertainty by virtue of sufficient data can then be the driver for very conservative risk assessment approaches.
Beyond state-of-the-art

For progress beyond the state of the art, the conventional Risk Assessment framework should be supplemented with non-conventional tools like Weight of Evidence (WoE) and Multi Criteria Decision Analysis (MCDA). In addition, a holistic (and if possible a probabilistic) approach should be explored for human health risk assessment (HHRA) and freshwater/terrestrial ecotoxicological risk assessment (FTERA) by bridging the gaps between the current state of the art and the conventional quantitative risk assessment approaches. This approach should involve a material life cycle perspective to enable comparison and aggregation of the health impact over the material’s life cycle stages. The applicability of a general approach to estimate the human effect factors for both linear and non-linear dose response relations for different health endpoints, or alternative developed indictors for hazard values, resulting in compatible output values of HHRA (e.g. different human health metrics) will need to be explored.

Risk Assessment

Since nanomaterials are extensively modified regarding surface-coating, size, shape, agglomeration state etc, it is extremely important to identify the physico-chemical properties that predict different toxicological outcomes, so that risk assessment of nanoparticles can be made on the basis of this information and using read-across to particles with similar physico-chemical characteristics. However, for NM, there is considerable debate surrounding the accuracy of reading across from other materials (e.g. the bulk form) and it is considered that this is not yet appropriate for NM without further study and validation. ENM are complex groups of materials with diverse physicochemical properties, which not only can affect their biological activities but also the underlying mechanisms of action. Using the ‘one size fit all’ approach in testing nanoparticles is therefore ignoring the complexity of the toxicity and mechanism of these nano-scale materials.

Needs by 2020:
• Validated predictive hazard/toxicity assessment methods for nanomaterials based on an understanding of the biological mechanism-of-action with a clarification of the association between material characteristics and toxic effects.
• Predictive assessment of toxic effects including inflammation, genotoxicity, immunotoxicity, carcinogenicity, cardiovascular toxicity and pulmonary toxicity etc.

• Development and validation of high throughput screening approaches to enable ‘high concern grouping approach’ with risk banding tools to identify potential hot spots for risk and to enable straightforward interpretation.

• Testing and calibration and further development of risk prioritization (or banding) tools.

• Development of a dermal risk prioritization/ banding tool.

**Risk management**

Currently, a suite of risk prioritization/ control banding tools has been developed to provide non-specialists with tools to decide on the need to manage a (potential) risk related to the use of nanomaterial/products. The (virtual) risk bands relate to levels of exposure control, according the hierarchy of control. However, for most of the control measures the effectiveness for nano particles has not been proven or quantified in practice.

Needs by 2020:

• Quantification of exposure reduction effectiveness of general and nano-specific control measures and strategies. Studies are warranted to examine the impact of ‘safety culture’ and human factors on the efficiency of control strategies.

• Calibration of risk/control banding tools, e.g. by expert evaluation.

**Risk governance: dialogue, communication, responsibility and trust**

To date, risk governance models have been conducted mainly on a ‘macro’ level, i.e. at the level of governments or even multinational bodies (Renn & Roco, 2006; IRGC, 2005; Roco et al., 2010). At the level of individual companies and organizations, it seems that the current activities that could be considered as being related to implementing a governance approach can be characterized as both scattered and ‘ad hoc’ (Dijkman & Terwoert, 2011). A comprehensive, structured approach appears to be missing in practically all cases, and it seems
that individual companies will need guidance in order to improve this situation, if they wish to counteract the potential threats to their business.

As far as communication and dialogue is concerned, many national and international initiatives for public dialogues have been started. Nonetheless, the involvement of specific target groups, such as workers, has remained modest. In general, it has been assumed that the perception of risks is influenced by whether the exposure to the risk is voluntary or involuntary (Sjöberg, 2000; Senjen & Foss Hansen, 2011). Experts and governments often believe that simply providing sufficient information will convince people that the benefits of a new technology outweigh its risks. This opinion assumes that the experts know the true risk, whereas often they do not. Few attempts have been made to involve workers in surveys of risk perceptions on nanotechnology. The attempts that have been undertaken have shown very low responses, perhaps due to a lack of awareness and a lack of perceived urgency (Van Broekhuizen et al., 2011).

Figure 4.16. Risk governance for nanotechnology: key activities. Adopted from International Risk Governance Council, White Paper on Nanotechnology No 2 (IRGC).
Current risk assessment and governance frameworks lack the ability to incorporate flexibility and learning, and arrangements to facilitate listening for early warnings. The same holds true for listening and responding to stakeholders’ concerns. They seem to overemphasize the role of technical experts and hence to disenfranchise the general public or workers, people who ought to be involved and have a voice in risk assessment and an input into risk management (Senjen & Foss Hansen, 2011). It is necessary to look at risk and risk assessment for nanotechnology in a much wider sense. Non-technical issues, such as monitoring stakeholders’ concerns, risk communication, involvement of and dialogue with stakeholders, making arrangements for the distribution of responsibilities and recording early warnings all have a role to play. Only if these aspects are regarded, the threats and barriers described may become mitigated. However, there is a major lack of practical guidance available for companies and other organisations on how to arrange proper governance of the uncertain risks associated with nanotechnologies, dealing with the non-technical aspects of risk management in addition to the technical aspects.

What appears to be needed is evidence-based, practicable, yet comprehensive guidance for companies and organisations, on the following aspects:

1) ‘Concern assessment’ - guidance on identifying stakeholders, their concerns, risk perceptions, norms, values and interests and arranging a dialogue on these issues.
2) ‘Concern management’ - guidance on channeling concerns raised during stakeholder dialogue on how to strive for acceptance of responsibilities, how to mitigate conflicts of interest and on risk communication strategies.

**Human field studies and epidemiology**

Epidemiological studies are a key tool for assessing whether exposed populations are safe at given levels, to evaluate the relevance of toxicological findings to human health and to identify potential biological effects that had not been predicted on the basis of toxicological tests.

Workers are likely to have earlier and higher exposures to ENMs than the general population, thus workers are a good target in which to conduct the first epidemiological studies.

It is recognized that conducting epidemiologic studies for ENMs is challenging for several reasons, such as the heterogeneity of particles and their
potential health effects, factors related to the assessment of exposure, the need to identify a population of workers with long-term exposure to ENMs especially those of appropriate size (Trout & Schulte, 2010). When dealing with new and emerging technologies such as nanotechnologies, it is not unreasonable to assume that epidemiological studies will also be needed in the future.

A major challenge for setting up human field studies is the identification of a sufficiently large number of workers with exposure to similar ENMs. Moreover, nanoparticles are likely to become more and more sophisticated because of intentional manipulation of the material at the nanoscale level (Maynard et al., 2011). Failure to account for particle heterogeneity can lead to a misclassification of the exposure. A key requirement for all epidemiological studies is good quality exposure assessment. Ideally, quantitative exposure assessment should provide sufficient information to estimate the intake or deposited dose of the substance under study in the individual workers.

**Identifying health effects from ENM and potential biomarkers**

Since specific health end points of ENMs are not known yet, studies on the chronic effects with adequate latency are probably not feasible in the near future. In contrast, initial epidemiological studies should focus on short-term effects and biomarkers of early effects in cross sectional and panel studies. Biomarkers of effect can be used in health surveillance programs aimed at the early diagnosis of exposure-related or associated diseases, but the application of effect monitoring is most often used to evaluate whether a well-characterized exposure can be associated with a shift in the distribution of relevant biochemical or functional endpoints indicative of early changes in the target or critical organs/tissue. For preventive purpose, biomarkers should not be considered as diagnostic tests but rather as indicators reflecting early modifications preceding progressive structural or functional damage at the molecular, cellular, and tissue level, i.e. changes possibly leading to adverse effects but completely reversible with the removal from the exposure. Therefore, there is a need for the selection of candidate biomarkers of early effects which can be used in human studies.

The relatively small current workforces in individual countries will probably necessitate the pooling of international cohorts. A targeted European multicenter study recruiting occupationally exposed workers and involving companies manufacturing and using ENM seems – at the moment – to be the most suitable way to attain a population with adequate size, homogeneous exposure type and
sufficient contrasts in exposure levels to permit exposure-effect relationships. To provide a coherent approach and make future epidemiological research a reality, a well-defined framework is needed for the careful choice of materials, exposure characterization, identification of study populations, definition of health end-points, and evaluation of the appropriateness of study designs, data collection and analysis, and interpretation of the results (Riediker et al., 2012).

While future studies should address the specificity of biomarkers, the priority is to evaluate whether quantitative changes in the already validated biomarkers occur in groups of exposed workers, and not to assess disease end-points. While sensitivity is fundamental for preventive purposes, specificity is usually more important for diagnostic purposes. Since nano-specific biomarkers are difficult to demonstrate, practical considerations suggest that it would be advisable to focus on the sensitivity to assess the causality of exposure conditions/scenarios and association with hazards.

**Databases**

Databases are crucial tools for storing and linking generated data. In nanosafety research, databases are used to link measurements of nanoparticle’s characteristics and their metrology protocols with the toxicology data.

To perform quality assessments on the available data, it is preferable to collect and store the data in a structured way specifically designed for risk assessment purposes. This approach can also provide guidance for future experimental setup to facilitate comparability of studies. The ultimate validation of data storage lies in the use of the data to predict behavior of ENMs, to assist in designing intelligent testing strategies, to provide advice to policy makers and regulators and to convince manufacturers to design low toxicity applications.

Currently, most FP7 projects in the NMP program have their own databases. For risk assessment (RA) purposes an overview of the current scientific status on all individual steps in the RA is essential. To facilitate such an overview there is a need for harmonization of data storage in projects. The prospect of harmonization of all databases generated by the FP7 NMP program (and other national projects in Europe and USA/Japan) is expected to facilitate a future inclusion of all information and the possibility of performing a meta-analysis on this comprehensible dataset to identify the Structure-Activity relationship and therefore move nearer to the vision of ‘Safety-by-Design’ for nanotechnology.
Due to the exponential growth of the nanotechnology literature, including the reports concerned about nanosafety, there is an on-going need to obtain an integrated quantitative perspective on the knowledge state of this literature. The most natural way to achieve this goal is to be able to automatically extract information from the published, peer-refereed scientific literature. Thus the creation a database in the field of nanosafety will involve collecting data from public domain sources and from internal ones (EU consortia) for automatic data mining and for allowing advanced search. This is the next step toward automating the relations between the nano data and toxicity. If one wishes to achieve this goal, it will be necessary to develop an ontology, which should explicitly represent the knowledge in the scientific domain of nanosafety.

Conclusions

To support the guidance in areas like risk management and decision-making, additional research will be needed in the fields of risk perception among many different targeted stakeholder groups and the main factors causing concerns.

The main achievement will be the development of integrated risk assessment and decision frameworks to enable forecasting the potential impacts of nanomaterials on human health and the environment and adequate risk management; this undertaking may require the development of novel risk assessment strategies that will replace the current one, being equally reliable, affordable but faster.
These achievements will be based on the following ‘building blocks’: Prediction of toxicity on the basis of physical/chemical properties of nanomaterials

- Models for release, fate and exposure to nanomaterials
- Integration of life cycle considerations into risk assessment
- Integration of risk assessment into decision framework of risk management
- Integration of safe-by-design, closed production-to-product and green nanotechnology approaches into the development stages of new nanomaterials and their applications

Key aspects of risk assessment and management
References


Pakarinen K, Petersen EJ, Leppänen MT, Akkanen J, Kukkonen JVK. Adverse effects of fullerenes (nC60) spiked to sediments in Lumbriculus variegatus (Oligochaeta). (2011) Environmental Pollution 159, 3750-3756.


Required research priorities to reach the goals of the roadmap

The roadmap for nanosafety research 2015-2025 aims to provide an understanding of where the European nanosafety research should be during the time frame 2015-2025. The roadmap also identifies the main achievements of this research which should have been reached during this time frame. This time horizon has been chosen based on the timing of the “Horizon 2020” Framework Programme for Innovation and Research; its first calls will open in 2014. The execution of the research of the first projects of the programme will start in 2015, and the implementation of their results around 2019-2020. The last calls of this programme will close in 2020 and the final projects will end around 2025, and the implementation of the results in full will start after that time point. The chosen window not only covers the time frame of the “Horizon 2020” funding Programme, but also takes into account the challenges associated with the prediction of expected results and research that will be required to reach the set goals.

The milestones in the roadmap indicate the expected achievements of nanosafety research at different time points, with 5-year intervals, during 2013-2025. These milestones are presented for the four thematic priority areas separately in four tables below, and have been described in detail in the previous chapters. In each table, the research priorities have been grouped under larger heading, topics, that cover several defined research priorities.

Within the topics the subheadings then provide the separate research priorities in the four thematic areas separately. All the topic areas and research priorities have been drawn from the topic areas and research priorities presented in the four chapters presented above 1) material characterization; 2) Exposure assessment and release during the life cycle; 3) Hazards, biokinetics, and vulner-
able populations; and 4) Risk prediction tools.

This roadmap on nanosafety research provides the vision of the Nano-Safety Cluster on strategic and emerging issues in the nanosafety research area. These are the topics in which progress will be crucial in the discovery of novel engineered nanomaterials and their nanotechnology applications. The roadmap is intended to guide the European Commission and also the national funding organizations when making strategic funding decisions on nanosafety research in the future.

The challenges in the prediction of the ability to achieve set research goals have been considered in compiling this document. It will be imperative to continuously follow the progress of the research and its impact on the emerging innovations as they are translated from laboratory to industry. This continuous assessment of the results of the priority research areas will be compared with their impact on industrial innovations and breakthroughs in nanosafety research. This document also emphasizes the potential of nanosafety research to nurture and support useful innovations that could lead progress in the understanding of key-features of safety and hazard mechanism of ENM. However, an equally important aspect is their ability to provide opportunities for creation of new types of consultative and service activities; many of these could expand and soon become a significant commercial activity similarly as ENM and nanotechnologies become incorporated into more and more business sectors.

In the following section, the milestones for 2014, 2020, and 2025, related, topics, and related research priorities under different topic separately are presented in a set of four tables.

Table 5.1. presents the roadmap, i.e. milestones 2015-2025, topics and research priorities in different topics at different time points under the thematic chapter on material characterization and grouping.
Table 5.1. Nanomaterial identification and classification

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material classification</td>
<td>Definition</td>
<td>Classification systems in place</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Naming structure</td>
<td>Ontologies in place</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Characterization of ENM in complex matrices</td>
<td>Robust methods for ENM size determination</td>
<td>Methods for ENM surface characterization</td>
<td>Methods for multicomposite ENM characterizaton</td>
</tr>
<tr>
<td></td>
<td>Test&amp; reference ENMs</td>
<td>Systematic sets of test ENMs</td>
<td>Full datasets on test ENMs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Validation</td>
<td>Validated labelled versions of test ENMs</td>
<td>Validation of key metrics for impact</td>
<td>Correlation of uptake, form and impacts</td>
</tr>
<tr>
<td>Measurement principles</td>
<td>Versatile methods</td>
<td>Versatile reference methods available.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-nano-interactions</td>
<td>Biomolecules for uptake, transport etc.</td>
<td>Reference bio-interactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NM impacts on protein function</td>
<td>Reference bio-interactions</td>
<td>NM properties leading to signalling disfunction</td>
<td></td>
</tr>
<tr>
<td>ENM engineering</td>
<td>Safety by design concepts</td>
<td></td>
<td>Safe design of new ENM in a bottom-up approach</td>
<td></td>
</tr>
<tr>
<td>ENM metrics for hazard assessment</td>
<td>Key descriptors</td>
<td>Non-spherical descriptors defined</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dose metrics</td>
<td>Effect of polydispersity in physico-chemical properties</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2. introduces the roadmap of the Thematic chapter on Hazard mechanisms, biokinetics and toxicity testing, i.e. the roadmap for 2015-2025, the topics revealing several associated research priorities under them, and then the actual research priorities for different time-frame separately.

Table 5.2. Hazard

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biokinetics and translocation</strong></td>
<td><strong>Prerequisites for research on ENM kinetics</strong></td>
<td>Nanomaterial-specific analytical equipment available</td>
<td>Biokinetics integrated into toxicological testing</td>
<td></td>
</tr>
<tr>
<td><strong>Hazard assessment</strong></td>
<td><strong>New approaches for ENM hazard assessment</strong></td>
<td>Developing systems biology approaches using omics technologies</td>
<td>Development of appropriate QSAR models</td>
<td>A computational tool that can assess in the predicting of ENM safety</td>
</tr>
<tr>
<td><strong>Vulnerable conditions</strong></td>
<td><strong>ENM and susceptible populations</strong></td>
<td>Systematic research of ENM with known disorders</td>
<td>Validated in vitro models of appropriate ENM</td>
<td>Validated in vivo and ex vivo models for different diseases</td>
</tr>
<tr>
<td><strong>Science-based regulatory approaches</strong></td>
<td><strong>Choice of test methods</strong></td>
<td>Improved strategies for testing</td>
<td>Intelligent testing strategies available</td>
<td>Regulation</td>
</tr>
</tbody>
</table>
Table 5.3 depicts the roadmap related to the Thematic Chapter on exposure and transformation of engineered nanomaterials, and presents the different topics areas covering a range of specific priorities which make the more detailed list of the priorities in this thematic area during 2015-2025.

Table 5.3. Exposure and transformation

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td>Release and exposure</td>
<td>Mechanistic understanding</td>
<td>Process knowledge to allow the set-up of realistic laboratory simulation</td>
<td>Database on emission (per time) and release (per material unit) factors</td>
<td>Understanding effects of ageing on nano-objects</td>
</tr>
<tr>
<td>Process dependent transformation</td>
<td>Transformation, mobility/transport</td>
<td>Gain knowledge on environmental mobility and transformation for computer simulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure scenarios</td>
<td>Workplace, consumer and environmental exposure</td>
<td>Exposure registries developed</td>
<td>Exposure models available</td>
<td>Models available for use of product cycle and exposure assessment</td>
</tr>
</tbody>
</table>
Table 5.4. introduces the Thematic Chapter on Risk prediction and management tools, and the relevant milestones to be achieved during 2015-2025, the wider topic areas under this thematic area, and the specific research priorities at different time points during 2015-2025.

### Table 5.4. The risk prediction and management tools

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk assessment</strong></td>
<td>Pro-active risk management</td>
<td>Risk banding tools/ effective control measures development</td>
<td>High throughput screening approaches validated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tools</td>
<td>Quantification of exposure reduction effectiveness</td>
<td>Testing and development of risk prioritization tools</td>
<td>RA-enabled LCA/ integration in decision tools</td>
</tr>
<tr>
<td><strong>Health</strong></td>
<td>Health effect</td>
<td>Markers for short term effect identified</td>
<td>Markers for long term effect identified</td>
<td>Implementation of the markers</td>
</tr>
<tr>
<td></td>
<td>Register</td>
<td>Health surveillance registries developed</td>
<td>Using registries for research</td>
<td>Implementation of results for regulations</td>
</tr>
<tr>
<td></td>
<td>Study design</td>
<td>Pilot panel studies completed</td>
<td>Case-control studies completed</td>
<td>Longitudinal studies started</td>
</tr>
<tr>
<td><strong>Databases</strong></td>
<td>Infrastructure</td>
<td>Federated databases available</td>
<td>Format &amp; data quality standards set</td>
<td>IT procedures for automatic uploading</td>
</tr>
<tr>
<td></td>
<td>Ontologies</td>
<td>Ontologies in place</td>
<td></td>
<td>Automation of ontologies</td>
</tr>
</tbody>
</table>
Table 5.4. The risk prediction and management tools (continued)

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Topic</th>
<th>By 2015</th>
<th>By 2020</th>
<th>By 2025</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk management</strong></td>
<td><em>Risk perception and guidance</em></td>
<td>Development of risk communication strategies</td>
<td>Guidance on stakeholder concern assessment</td>
<td>Guidance on risk evaluation</td>
</tr>
<tr>
<td></td>
<td><em>Prevention through design approach</em></td>
<td></td>
<td></td>
<td>Integration of safe-by-design approaches into the development stages of new nanomaterials and their applications</td>
</tr>
</tbody>
</table>
Table 5.5. summarizes at a more general level the main milestones for different thematic areas are presented in parallel to allow a general level comparison of the main milestones in these thematic areas, and identification of their interrelationships so that they become apparent for the reader, and allow him/her to obtain a more general understanding of the strategic goals of the research priorities proposed in this document.

Table 5.5. Summary of thematic roadmap and research priorities

<table>
<thead>
<tr>
<th>Time</th>
<th>Material</th>
<th>Exposure</th>
<th>Hazard</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Reference methods and nano-bio-interactions</td>
<td>Laboratory and computer simulations</td>
<td>Systems biology approaches available for hazard research</td>
<td>Improved risk communication and tools for risk assessment</td>
</tr>
<tr>
<td>2020</td>
<td>Data sets on reference ENM</td>
<td>Database on release</td>
<td>Understanding the association between material characteristics and hazard</td>
<td>Models and standards available</td>
</tr>
<tr>
<td>2025</td>
<td>Key metrics for harmful impact</td>
<td>Laboratory tests and models available for exposure assessment</td>
<td>A tool for safety assessment</td>
<td>A tool for the integration of safety by design strategies</td>
</tr>
</tbody>
</table>
We have listed some practical steps to achieve the goals of this roadmap and research priorities on which the roadmap is built:

1. Understanding the properties of both nanomaterials and cells or organisms, driving the nano-bio interactions.
2. Generate the consciousness and the tools to develop the concept of “safe by design” to be applied in the generation of new materials and devices targeting both industry and scientific world.
3. Technical development to allow more accurate measurements of nanoparticles under realistic exposure condition, in situ in media like soil, consumer products and food.
4. Develop guidelines on how to determine a nanomaterial including easy to use instruments and agreed measurement protocols. Develop agreed descriptors for complex non-spherical structures.
5. Develop instruments to separate engineered from background nanomaterials.
6. Develop dose-response relationships for the identified relevant descriptors / parameters.
7. Master the nanosafety issues of the 1st generation materials.
8. Tackle the issues associated with the second and third generation of nanomaterials and complex mixtures entailing nanomaterials. Stability and degradability as well as monitoring tools must be established.
9. Develop the tools for proper risk assessment on the above mentioned issues, specially the Life Cycle analysis, where the nanosafety issues of nanomaterials are considered from synthesis and fabrication to their end of life.
6 Implementation of the roadmap

Within the last 10-15 years a number of novel basic methods to explore ENM-induced environmental health and safety (EHS) effects have been developed and validated. The implementation strategy proposed here is based on this existing knowledge. To facilitate and to enhance the advancement of nanotechnology, a successful implementation of a comprehensive and implementable scientific research agenda is of utmost importance.

The most important key for a successful implementation is the excellence of the research proposed aimed at meeting well identified and justified priorities of the Strategic Research Agenda (SRA). The SRA shall be realistic in terms of goals and contents. Additional key elements of a successful implementation of such SRA for nanosafety research have been summarised in the key topics identified in the report of the National Research Council (NRC 2012; http://www.nap.edu/catalog/).

- Key audiences for implementing the strategy
- Infrastructure for implementation and accountability
- Evaluation of research progress and revision of the strategy

Resources to conduct research and suitability of the SRA to be implementable within the framework of horizon

A successful implementation of the SRA on nanosafety is based on the full integration of all stakeholders in deciding the priorities to be pursued in the research agenda, in the transfer of research results into applications and safety standards and in the evaluation of the progress achieved based on the goals of the SRA.
This integration requires one or more overarching bodies to facilitate the exchange of information, discussion and decision making. Stakeholders to be involved in these decision making bodies are small-to-medium-sized enterprises, industry in general, political decision makers, regulators, occupational and other hygienists, safety personnel and the scientific community.

Some of these stakeholders are organised in larger organisations working either on a European or a global scale such as European Chemicals Agency (ECHA), European Food Safety Authority (EFSA), OECD, ISO, CEN, WHO, NGO’s. These organizations need to become directly committed and included in the implementation of the SRA to avoid duplication of research, as well as providing valuable input in setting priorities and ensuring optimal and appropriate application of the fruits of the research efforts.

**Infrastructure for implementation and accountability**

It is quite clear that the implementation of the SRA it is crucial demands an effective infrastructure in order to ensure that the proposed milestones are achieved on time and on budget. Some initiatives to support the establishment such infrastructures already exist. These include the NanoSafety Cluster and NANOfuture. However, overarching platforms for exchange information between industry, decision makers, regulators, academic community and politicians are still lacking. Not all stakeholders and groups of stakeholders are aware of the nanosafety research needs and progress being made in this area. To enable the accountability of such platforms, it must be a part of the bodies/organizations executing the SRA. The different dimensions of accountability which need to be managed are summarised in the report of NRC (2012):

- Ensuring and assigning ownership of the overall strategy.
- Establishing appropriate means of governance among parties implementing the strategy (an overarching body, exchange platform integrating all stakeholder interests has to be formed).
- The above point can be related to the proposal made in the chapter on infrastructures required to support nanosafety research, notably the establishment of a virtual European Nanosafety Research Centre with competence entry criteria with one of the parties of this network coordinating this activity; the centre’s activities should be governed
by the representatives from all of the participating organizations.

- Establishing and applying mechanisms for accomplishing exploratory, translational, and targeted research in the context of the strategy, including an appropriate balance between government and private-sector funding and facilitating needed or desired interdisciplinary research. Such activities could be coordinated by the above mentioned European Virtual Nanosafety Research Centre proposed in the chapter on infrastructure.

- Assigning responsibility for executing elements of the strategy (e.g. SRA executed by members of the NSC). Implementation of the SRA could be supported by the Virtual European Nanosafety Research Centre described above together with the NSC with shared responsibilities.

- Ensuring that stakeholders are involved in the activities of SRA and that they have substantial input into formulating and reviewing these activities.

- Monitoring of progress of the identified research priorities and timelines to ensure that the strategy is conducted effectively and efficiently and to ensure that the responsible parties are held accountable for the research progress.

- Coordinating periodic review and revisions to the strategy.

- Ensuring that sufficient resources are devoted to undertaking the defined research and to implementing the overall strategy and allocating and managing the resources.

Some of the above bullet points have been annotated in accordance to a system already in operation and which is envisaged to be implemented by the NSC in the SRA. Nevertheless, open issues still remain to be solved for a full implementation of the SRA.

**Evaluation of research progress and revision of the strategy**

It is important that an independent research panel e.g. formed by the NanoSafety-Cluster will review the advances after 2-3 years and will compare the results with the research roadmap presented here. This review will then facilitate the update and possibly demand some modifications to the research plans and calls.
The review has to be discussed in an open platform including all stakeholders. Facilitation and funding of this activity, thus far limited to European research, has to be made e.g. in form of CSAs funded by the EU Commission.

**Resources needed to conduct research and suitability of the SRA to be implementable within the framework of horizon 2020**

The task of promoting and facilitating the implementation of the SRA lies with the NSC, their working groups and the European research projects. A clear commitment to this role of the NSC, their task and some basic funding will be needed to enable the implementation and review of the progress. Ensuring a stable structure such as the earlier mentioned Virtual European Nanosafety Research Centre, a network of dedicated nanosafety research organizations, would add stability to these undertakings.

**Different types of research within the frame of the Strategic Research Agenda for Nanosafety Research 20145-2025**

The development of research within the framework of Horizon 2020 can be generally divided into Breakthrough Innovation Research, Research in Support of Regulation, and Research in Support of the Market. This also applies to the environmental, health and safety (EHS) issues for nanotechnologies, nanomaterials and products based on nanomaterials. It is also important to differentiate those EHS tools which are developed and applicable independent of a specific product or production/processing process and those which are specifically developed or adapted to specific needs.

Under the heading **breakthrough innovation** it is important to extend the detailed knowledge we have gained in the recent years to achieve a more generalised understanding and better models allowing the prediction of behaviour, exposure, hazard and risk. Tools for driving this innovation are mainly the Research Framework Programme and nanosafety funding programmes in the EU Member States for the next years to come. The research is complemented, as emphasized above, by specific projects such as the SIINN ERANET II, and by national activities of all EU Member States. Topics to be dealt with in break-
through innovation include the developments of equipment and skills necessary to be in place, or a stable network of organizations, when the new technologies reach the market for the specific applications in the different industrial sectors. This includes all exposure pathways, such as workers, consumers or exposure via the general environment as well as distinct environmental compartments.

The main priority themes identified by the NSC SRA include the following topics:

- Material identification and classification
- Release, exposure and transformation
- Hazard mechanisms and biokinetics
- Risk assessment and managements

The above priority themes include several sub-headers which have been more specifically explained and prioritized in the corresponding sections.

To be prepared to cope with imminent challenges, the following topics are considered by the NSC to be of importance and need to be dealt with in the next few years:

- Building a testing and modelling framework for nanomaterial release potential into the environment
- Modelling environmental transformation, transport and fate of nanomaterials
- Keeping pace with innovations by ensuring their nanosafety; development of next generation tools for risk governance of existing and next generation nanomaterials
- High Throughput and Toxicity Pathway approaches as a basis for nanosafety assessment, nanomaterial grouping and read-across strategies
- Establishing advanced and realistic in vitro models for nanomaterial toxicity testing
- Establishing extrapolation from in vitro to in vivo (animals, man) and vice versa
- Utilizing material characterization techniques, and identifying association of material characteristics or bio-identity with hazardous effects of ENM with living organisms
- Utilization of these research outputs to develop a more affordable, reliable and quicker paradigm for the assessment and management of risks of ENM

The above topics are major building blocks in creating a more complete EHS tool for the sustainable implementation of nanotechnologies and products. The projects are mainly seen as large R&D projects coordinated on European scale. The tools developed in the first years will end in model understanding and applicable models. The research envisaged for 5-10 years will combine the information of the first years into a set of modelling and prediction tools needed for better risk assessments, risk management and risk governance. NanoEHS issues should further be integral parts in R&D activities directed towards new nanotechnology related production and products. By cross-linking the general development activities closely with technology developments the highest degree of information transfer can be achieved. NSC clearly recommends that such cross-linking should be an obligatory part in any nationally or internationally funded activity.

Another important building block to facilitate the safe implementation of nanotechnologies is research in support of regulation, and its implementation is crucial for strongly promoting nanosafety within EU in the near future. This research is intended to provide science based regulatory approaches to ensure societal acceptance of nanotechnologies and good understanding between authorities, industry and society about risks and their management.

It is also important to encourage the EU Member State Governments to support applied research with a short time horizon that emphasizes the safety of products already on the market. This research in support of the market is important in short term. This market-oriented research should include:

- Support of “safe markets”: knowledge networks / transfer to industry / epidemiological cohorts / certification / training and skills / benchmarking / best practice
- Defining and quantifying acceptable risk / Risk communication / Risk management
Conclusions

This research roadmap aims at providing directions towards a sustainable development of nanotechnology based tools and products. It is based on the premises that a level of generalised knowledge in the different areas mentioned and dealt with above shall be achieved within the next 10-15 years and this will mean that new materials will be safer by design and this philosophy will be beneficial not only for Europe but for the whole world.
Annex: Editorial group and list of contributors

The Editorial Group of the “Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations” was given the task to finalize the thematic chapters, the introduction and research landscape chapters, the cross-cutting issues chapters, and to provide conclusions, recommendations and executive summary for the document to be finally approved by the NanoSafety Cluster. It was also agreed that the work of every individual who contributed to the document would be carefully recorded.

At the beginning of the work on in 2011 fourteen working groups were appointed to explore and identify the most topical issues for the future research on the safety of ENM and products incorporating them. In this work among other the following priority issues were identified: 1) material characterization; 2) exposure assessment; 3) human and ecotoxicology; 4) risk assessment; 5) disease models; 6) databases and ontologies; 7) mathematical modelling; 8) risk communication tools; and 9) dissemination and impact. It was soon recognized that further condensation of the fourteen working groups was needed merging into large entities to allow better prioritization of the research topics. The NanoSafety Cluster appointed an Editorial Group consisting of a chair and chairs of the four thematic groups that were merged from the initial fourteen working groups. The four merged topic areas were: 1) material identification and classification (chair Dr. Iseult Lynch); 2) exposure and transformation (chairs Dr. Ulrika Backman and Dr. Thomas Kuhlbusch); 3) hazard mechanisms including both human toxicology and ecotoxicology (chair Dr. Teresa Fernandes); and 4) risk prediction tools including databases and ontologies (chair Dr. Derk Brouwer). Additionally, the chair of the Editorial group was Dr. Kai Savolainen as the coordinator of the NanoSafety Cluster, and the other appointed members were Dr. Bengt Fadeel and Dr. Robert Landsiedel.

List of contributors in alphabetical order:

Ahluwalia Arti, University of Pisa, Italy (arti.ahluwalia@ing.unipi.it)
Ahtiainen Jukka, Finnish Safety and Chemicals Agency, Finland (jukka.ahtiainen@tukes.fi)
Aitken Robert, Institute of Occupational Medicine (IOM), UK and IOM Singapore, Singapore (rob.aitken@iom-world.org)
Annex: Editorial group and list of contributors

Alenius Harri, Finnish Institute of Occupational Health, Finland (harri.alenius@ttl.fi)

Asbach Christof, Institute of Energy and Environmental Technology,
    Germany (asbach@iuta.de)

Athersuch Toby, Imperial College London, United Kingdom
    (toby.athersuch@imperial.ac.uk)

Backman Ulrika, Technical Research Centre of Finland, Finland
    (ulrika.backman@vtt.fi)

Bergamaschi Enrico, Dept. of Clinical and Experimental Medicine,
    University of Parma, Italy (enrico.bergamaschi@unipr.it)

Berges Markus, Institut für Arbeitsschutz der
    Deutschen Gesetzlichen Unfallversicherung, Germany (markus.berges@dguv.de)

Bessems Jos, National Institute for Public Health and the Environment (RIVM),
    The Netherlands (jos.bessems@rivm.nl)

Bleeker Eric, National Institute for Public Health and the Environment (RIVM),
    The Netherlands (eric.bleeker@rivm.nl)

Bloch Daniel, French Alternative Energies and Atomic Energy Commission (CEA),
    France (daniel.bloch@cea.fr)

Boland Sonja, Université Paris Diderot-Paris, France (boland@univ-paris-diderot.fr)

Boraschi Diana, Institute of Biomedical Technologies, National Research Council,
    Italy (diana.boraschi@gmail.com)

Bos Peter, National Institute for Public Health and the Environment (RIVM),
    The Netherlands (peter.bos@rivm.nl)

Bouillard Jaques, INERIS, France (Jaques.BOUILLARD@ineris.fr)

Boutou-Kempf Odile, Institut de vielle sanitarie, France (odile.boutou@ars.sante.fr)

Bouwmeester Hans, RIKILT - Wageningen University and Research Centre,
    The Netherlands (hans.bouwmeester@wur.nl)

Brouwer Derk, Netherlands Organisation for Applied Scientific Research (TNO),
    The Netherlands (dick.brouwer@tno.nl)

Byrne Hugh J., Dublin Institute of Technology, Ireland (hugh.byrne@dit.ie)

Buttino Isabella, ISPRA Ecotoxicology and Plankton Biology Lab, Italy
    (isabella.buttino@ispramiente.it)

Caillard Bastien, EU-Vri European Virtual Institute for Integrated Risk Management
    (bastian.caillard@eu-vri.eu)

Carlander David, Nanotechnology Industries Association, Belgium
    (david.carlander@nanotechia.org)

Calzolai Luigi, Joint Research Center of the European Commission, Institute for
    Health and Consumer Protection (luigi.calzolai@jrc.ec.europa.eu)

Cassee Flemming, National Institute for Public Health and the Environment (RIVM),
    The Netherlands (flemming.cassee@rivm.nl)

Cattaneo Stefano, CSEM Centre Suisse d’Electronique et de Microtechnique SA
    (stefano.cattaneo@csem.ch)

Costa Anna Luisa, Nanotechnologies and Colloidal Processing CNR - Institute of
    Science and Technology for Ceramics FAENZA (RA) - ITALY (anna.costa@istec.cnr.it)
Annex: Editorial group and list of contributors

Crossley Alison, University of Oxford, United Kingdom
(alison.crossley@materials.ox.ac.uk)

Dahmann Dirk, Institut für Gefahrstoff-Forschung, Germany (dahmann@igf-bbg.de)

Dawson Kenneth, University College Dublin, (Kenneth.A.Dawson@cbni.ucd.ie)

Dekkers Susan, National Institute for Public Health and the Environment (RIVM),
The Netherlands (susan.dekkers@rivm.nl)

Dondero Francesco, Università del Piemonte Orientale Vercelli Novara Alessandria,
Italy (francesco.dondero@unipmn.it)

Fadeel Bengt, Karolinska Institutet, Sweden (bengt.fadeel@ki.se)

Falk Andreas, BioNanoNet Forschungsgesellschaft mbH, Austria
(andreas.falk@bionanonet.a)

Fernandes Teresa, Heriot-Watt University, United Kingdom (t.fernandes@hw.ac.uk)

Gehr Peter, Institut für Anatomie University of Bern, Switzerland (gehr@ana.unibe.ch)

González Fernández África, Biomedical Research Centre (CINBIO),
University of Vigo, Spain (africa@uvigo.es)

Groenewold Monique, National Institute for Public Health and the Environment
(RIVM), The Netherlands (monique.groenewold@rivm.nl)

Gutleb Arno, Centre de Recherche Public - Gabriel Lippmann, Luxembourg
(gutleb@lippmann.lu)

Haase Andrea, Federal Institute for Risk Assessment, Berlin, Germany
(andrea.haase@bfr.bund.de)

Handy Richard, Plymouth University, UK (R.Handy@plymouth.ac.uk)

Hassellöv Martin, University of Gothenburg, Sweden (martin.hassellov@chem.gu.se)

Hazebruck Benoît, EU-Vri European Virtual Institute for
Integrated Risk Management (bh@eu-vri.eu)

Hristozov Danail, University Ca’ Foscari Venice, Italy (danail.hristozov@unive.it)

Hund-Rinke Kerstin, Fraunhofer Institute for Molecular Biology and Applied Ecology,
Germany (kerstin.hund-rinke@ime.fraunhofer.de)

Ilves Marit, Finnish Institute of Occupational Health, Finland (marit.ilves@ttl.fi)

Jensen Keld A., National Research Center for the Working Environment,
Denmark (kaj@nrcwe.dk)

Johnson Andrew, Centre for Ecology & Hydrology, United Kingdom (AJO@ceh.ac.uk)

Jovasevic-Stojanovic Milena, University of Belgrade, Serbia
(milena.jovasevic.stojanovic@gmail.com)

Juntunen Elina, Finnish Institute of Occupational Health, Finland (elina.juntunen@ttl.fi)

Kendall Kevin, University of Birmingham, United Kingdom (k.kendall@bham.ac.uk)

Koivisto Joonas, Finnish Institute of Occupational Health, Finland
(joonas.koivisto@ttl.fi)

Koppen Gudrun, Vlaamse Instelling voor Technologisch Onderzoek n.v. (VTTO NV),
Belgium (gudrun.koppen@vito.be)

Korenstein Rafi, Tel Aviv University, Israel (omni@eng.tau.ac.il)

Krombach Fritz, Institute for Surgical Research Ludwig-Maximilians-Universität,
München, Germany (krombach@med.uni-muenchen.de)
Kuhlbusch Thomas A.J., Air Quality & Sustainable Nanotechnology, IUTA e.V., Duisburg, Germany (tky@iuta.de)
Kühnel Dana, Helmholtz Centre for Environmental Research – UFZ, Germany (dana.kuehnel@ufz.de)
Landsiedel Robert, BASF Aktiengesellschaft, Germany (robert.landsiedel@basf.com)
Larese Filon Francesca, University of Trieste, Italy (larese@units.it)
Lofts Steve, Lancaster Environment Centre, United Kingdom (s.lofts1@lancaster.ac.uk)
Loureiro Susana, Departamento de Biologia & CESAM, Universidade de Aveiro
Lynch Iseult, University of Birmingham, United Kingdom (i.lynch@bham.ac.uk)
Maimon Oded, Tel Aviv University, Israel (maimon@eng.tau.ac.il)
Marcomini Antonio, University Ca’ Foscari Venice, Italy (marcom@unive.it)
Marvin Hans J.P., RIKILT Institute of Food Safety, The Netherlands (Hans.Marvin@wur.nl)
Migliore Lucia, University of Pisa, Italy (l.migliore@geog.unipi.it)
Nelissen Inge, Vlaamse Instelling voor Technologisch Onderzoek n.v. (VITO NV), Belgium (inge.nelissen@vito.be)
Njuguna James, Cranfield University, United Kingdom (j.njuguna@cranfield.ac.uk)
Norppa Hannu, Finnish Institute of Occupational Health, Finland (Hannu.Norppa@ttl.fi)
Oomen Agnes, National Institute for Public Health and the Environment (RIVM), The Netherlands (agnes.oomen@rivm.nl)
Pakarinen Kukka, University of Eastern Finland, Finland (kukka.pakarinen@uef.fi)
Palmqvist Annemette, Roskilde University, Denmark (apalm@ruc.dk)
Papadopoulos Manthos, Institute of Organic and Pharmaceutical Chemistry National Hellenic Research Foundation, Greece (mpapad@eie.gr)
Park Margriet, National Institute for Public Health and the Environment (RIVM), The Netherlands (margriet.park@rivm.nl)
Peijnenburg Willie, National Institute for Public Health and the Environment (RIVM), The Netherlands (willie.peijnenburg@rivm.nl)
Peters Ruud, RIKILT – Institute of Food Safety, The Netherlands (ruudj.peters@wur.nl)
Pietroiuisti Antonio, University of Rome, Italy (pietroiu@uniroma2.it)
Pozzi Micelli Stefano, Veneto Nanotech, Padova (stefano.pozzimicelli@venetonanotech.it)
Pronk Anjoeka, Netherlands Organisation for Applied Scientific Research (TNO), The Netherlands (Anjoeka.Pronk@tno.nl)
Pylkkänen Lea, Finnish Institute of Occupational Health, Finland (lea.pylkkanen@ttl.fi)
Rebe Sabina Raz, RIKILT – Institute of Food Safety, The Netherlands (sabina.rebe@wur.nl)
Riediker Michael, Institute for Work and Health, Switzerland and IOM (Institute of Occupational Medicine), Singapore (michael.riediker@iom-world.sg)
Rietveld Anton, National Institute for Public Health and Environment (RIVM), The Netherlands (anton.rietveld@rivm.nl)
Sachse Sophia, Cranfield University, United Kingdom (s.sachse@cranfield.ac.uk)
Salvi Olivier, EU-Vri European Virtual Institute for Integrated Risk Management (salvi@eu-vri.eu)
Annex: Editorial group and list of contributors

Sánchez-Jiménez Araceli, Institute of Occupational Medicine, United Kingdom  
(Araceli.Sanchez@iom-world.org)

Santamaria Jesus, Aragon Nanoscience Institute & Department of Chemical and  
Environmental Engineering, Spain (Santamaria@unizar.es)

Savolainen Kai, Finnish Institute of Occupational Health, Finland (kai.savolainen@ttl.fi)

Schlich Karsten, Fraunhofer Institute for Molecular Biology and Applied Ecology,  
Germany (Karsten.Schlich@ime.fraunhofer.de)

Scott-Fordsmand Janeck, Aarhus University, Denmark (jsf@dmu.dk)

Selck Henriette, Roskilde University, Denmark (selck@ruc.dk)

Sips Adrienne, National Institute for Public Health and the Environment (RIVM),  
The Netherlands (adrienne.sips@rivm.nl)

Sirviö Sari, Finnish Institute of Occupational Health, Finland (sari.sirvio@ttl.fi)

Stamm Hermann, Joint Research Center of the European Commission, Institute for  
Health and Consumer Protection (Hermann.stamm@ec.europa.eu)

Steinfeldt Michael, University of Bremen, Germany (mstein@uni-bremen.de)

Stockmann-Juvala Helene, Finnish Institute of Occupational Health, Finland  
(helene.stockmann-juvala@ttl.fi)

Svendsen Claus, Centre for Ecology & Hydrology, United Kingdom (csv@ceh.ac.uk)

Tielemans Erik, Netherlands Organisation for Applied Scientific Research (TNO),  
The Netherlands (Erik.Tielemans@tno.nl)

Tran Lang, Institute of Occupational Medicine, United Kingdom  
(lang.tran@iomhq.org.uk)

Valsami-Jones Eugenia (Éva), University of Birmingham,  
UK (e.valsamijones@bham.ac.uk)

Van de Meent Dik, National Institute for Public Health and the Environment (RIVM),  
The Netherlands (dik.van.de.meent@rivm.nl)

van Gestel Kees, VU University Amsterdam, The Netherlands (kees.van.gestel@vu.nl)

Vandebril Rob, National Institute for Public Health and the Environment (RIVM),  
The Netherlands (rob.vandebril@rivm.nl)

Vanhala Esa, Finnish Institute of Occupational Health, Finland (esa.vanhala@ttl.fi)

Vázquez-Campos Socorro, LEITAT Technological Center, Spain  
(svazquez@leitat.org)

Vippola Minnamari, Tampere University of Technology, Finland  
(minnamari.vippola@tut.fi)

Vogel Ulla, National Research Center for the Working Environment, Denmark  
(ulla.vogel@nrcwe.dk)

von der Kammer Frank, University of Vienna, Austria (frank.kammer@univie.ac.at)

Wick Peter, Swiss Federal Laboratories for Materials Science and Technology (EMPA),  
Switzerland (Peter.Wick@empa.ch)

Winther-Nielsen Margrethe, DHI, Denmark (mwn@dhi-group.com)

Özgüz Volkan, Sabanci University, Nanotechnology Research and Application Center,  
Istanbul, Turkey (volkan@Sabanciuniv.edu)